Abstracts

Third North American Conference on Mycorrhizae

August 23-25, 1977
Athens, Georgia

THIRD NORTH AMERICAN CONFERENCE ON MYCORRHIZAE

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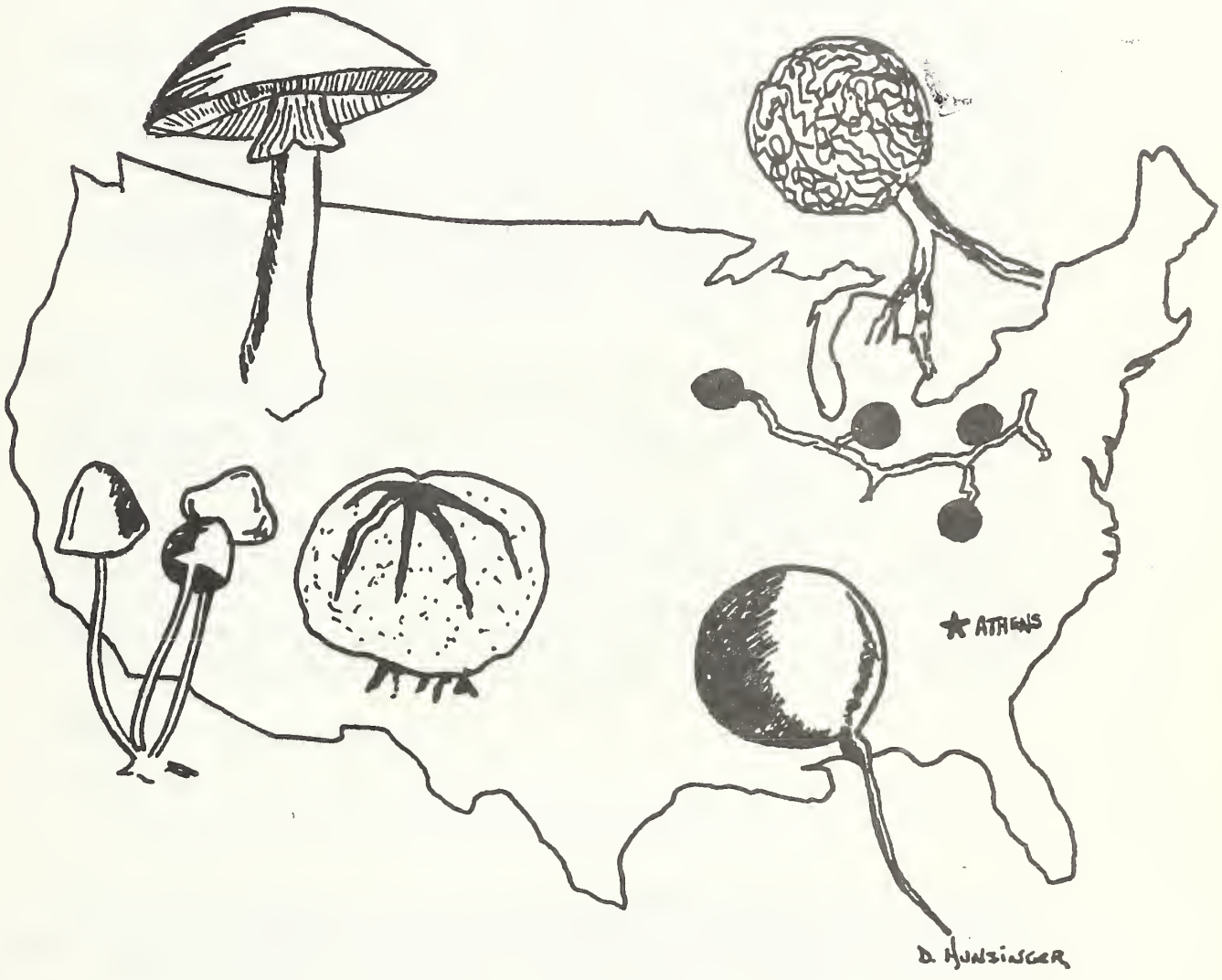

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PREFACE

Research on mycorrhizae has been in progress in North America since the early 1900's. Between 1950 and 1970 research on the role of mycorrhizae in plant nutrition and diseases was very intense. Many facets of the interactions of symbiotic fungi and plants were revealed during these periods . . . unfortunately, in many instances the only people interested in the findings were other researchers in the mycorrhizal field. Since 1970, however, not only have more people become interested in mycorrhizae, they have begun to appreciate the potential value of mycorrhizae to world production of food, fuel and fiber. The abstracts herein best demonstrate this new appreciation for mycorrhizae. The abstracts cover research accomplished on many diverse plant species carried out throughout North America and other parts of the world. Many cover research areas that are on the verge of practical application.

These abstracts were contributed by mycorrhizal researchers and organized into subject areas by the Chairman of the Conference. The abstracts were accepted without review or editing and should not be considered complete, published works. The author(s) of each abstract is responsible for content. Undoubtedly, research discussed in many of the abstracts will be published in journals. It is the recommendation of the Chairman that permission be obtained from the author(s) before citing information contained in the abstracts. Abstracts may be acknowledged as a personal communication between the author(s) and the individual.

DONALD H. MARX, Chairman
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TABLE OF CONTENTS

PRACTICAL APPLICATION OF MYCORRHIZAL TECHNOLOGY - Session I

PRACTICAL APPLICATION OF MYCORRHIZAL TECHNOLOGY - Session II

MYCORRHIZAE AND POLLUTANTS

FIELD OBSERVATIONS ON MYCORRHIZAL ASSOCIATIONS AND THEIR FUNGI

MYCORRHIZAE AND PLANT ECOLOGY

PHYSIOLOGY OF ENDOMYCORRHIZAL ASSOCIATIONS AND THEIR FUNGI

THE RESISTANCE OF VESICULAR-ARBUSCULAR ONION MYCORRHIZAE TO PYRENOCHAETA TERRESTRIS.

William N. Becker and James W. Gerdemann, Department of Plant Pathology, University of Illinois, Urbana 61801.

Vesicular-arbuscular mycorrhizae of an onion variety susceptible to Pyrenochaeta terrestris were more resistant to the pink-root pathogen than nonmycorrhizal onion roots. In plastic root chambers, selected onion root segments were directly challenged with P. terrestris. Yellow (mycorrhizal) roots generally stayed yellow but white (nonmycorrhizal) roots usually turned pink during the challenge period. Mycorrhizal roots had significantly fewer cortical cell layers penetrated by P. terrestris than nonmycorrhizal roots. The growth of the pathogen appeared to be restricted by cell wall thickening.

INTERACTION OF PRATYLENCHUS BRACHYURUS AND AN ENDOMYCORRHIZAL FUNGUS ON COTTON

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Little is known about the interaction of plant-parasitic nematodes and endomycorrhizal root symbionts even though they commonly occur together in the roots or rhizosphere of the same plant. We investigated the interaction of a migratory endoparasitic nematode, Pratylenchus brachyurus (PB), with a vesicular-arbuscular endomycorrhizal fungus, Gigaspora margarita (GM), on cotton (Gossypium hirsutum 'Coker 201') at two different fertility levels (equivalent to 560 and 1,120 kg/ha of 10-10-10 N-P-K) in the greenhouse. Treatments consisted of single inoculations with PB (5,000 nematodes/plant) or GM (250 azygospores/plant), joint inoculations, and appropriate controls. The effect of fertility level was evaluated for each of the treatments. Although the high fertility level increased cotton growth and reproduction when measured 77 days after transplanting, the greatest stimulation in plant development occurred when plants were inoculated with GM at each fertility level. At the low fertility, GM increased shoot height, fresh shoot weight and root weight, and square production 96%, 553%, 358%, and 760%, respectively, over that of nonmycorrhizal controls. Plant development was also stimulated by GM at the high fertility level, but the magnitude of the increase was not as great as at the low fertility rate. Even though cotton was a good host for PB, plant development was not retarded by the nematode at either fertility level. In concomitant culture, mycorrhizal-induced plant growth and reproduction, and sporulation of the fungus were not affected by the parasitic activities of PB on the cotton roots. Mycorrhizal synthesis in the cotton roots, however, suppressed the number of PB in root tissue.

The effect of initial sequence of infection on the interaction between *Glomus macrocarpus* and *Meloidogyne incognita* on soybean.

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Greenhouse tests were conducted to evaluate the effect of initial sequence of infection on the interaction between *Glomus macrocarpus* and *Meloidogyne incognita* on 'Pickett' soybean, a cultivar susceptable to root knot nematode. Pregerminated soybean seedlings were exposed to either 25 chlamydospores, 100 nematode eggs, or both in an aluminum foil funnel containing 150 ml of autoclaved sandy soil. Ten days later, after initial infection had occurred, plants were transferred to funnel shaped depressions made in autoclaved sandy soil in a 15 cm plastic pot. Prior to transplant, 25 chlamydospores, 100 nematode eggs, or both were placed around the sides of the depression. The experiment was repeated twice, once with four replicates and once with five replicates. Results indicated that prior colonization by *G. macrocarpus* did not significantly influence the initial number of galls produced by subsequent inoculation with *M. incognita* and prior infection by *M. incognita* did not significantly influence subsequent colonization by *G. macrocarpus*. Top weight, root weight, number of galls, number of chlamydospores, and percent of mycorrhizal roots were not significantly different between dually or sequentially inoculated plants and singly inoculated controls examined three, five, and eight weeks after planting. In data collected at eight weeks, the average number of galls in plants singly inoculated with *M. incognita* (9.3 galls/plant) was similar to the value obtained by averaging data from all plants inoculated with both *G. macrocarpus* and *M. incognita* including all sequences of inoculation (9.7 galls/plant) and the average number of chlamydospores produced on plants singly inoculated with *G. macrocarpus* (106.5 spores/100 ml of soil) was greater than the value obtained by averaging data from all plants inoculated with both *G. macrocarpus* and *M. incognita* including all sequences of inoculation (69.0 spores/100 ml of soil). Although these differences were not significant, placing *G. macrocarpus* and *M. incognita* together at planting resulted in greater root weight, a greater percent of mycorrhizal roots, more chlamydospores, and more galls per plant than resulted in singly inoculated controls and all other sequential treatments.

VESICULAR -ARBUSCULAR FUNGI AND THE PINK ROOT ORGANISM ON ONIONS; AN INTERACTION STUDY. Anne-Cressey McGraw, J.C. Zalewski, Dept. of Botany and Plant Pathology, Oregon State University, Corvallis 97330

A field survey of commercial onion fields in Eastern Oregon revealed low levels of vesicular-arbuscular (VA) fungi and high levels of the pink root organism, Pyrenochaeta terrestris (Hansen) Gorenz, Walker, and Larson, in "old" (cropped 20-25 years) soils, as compared to high levels of VA fungi and low levels of P. terrestris in "new" (2 years out of desert) soils. The observed differences in mycorrhizal associations may be due to differences in soil fertility of old and new onion fields. A greenhouse study to test the effects of Glomus fasiculatus and G. mosseae on infection of onions by P. terrestris is in progress. It is hypothesized that these VA fungi may afford the onion plant some protection from P. terrestris.

INTERACTION OF THE ENDOMYCORRHIZAL FUNGUS GIGASPORA MARGARITA AND ROOT KNOT NEMATODE ON COTTON

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Inoculation of cotton (Gossypium hirsutum) 'McNair 511' and 'Stoneville 213' with azygospores of Gigaspora margarita in greenhouse studies significantly increased vegetative growth and square production over nonmycorrhizal plants. Growth stimulation and plant development was greater at a low fertility rate (250 ppm 10-10-10) than at a high fertility level (500 ppm). Shoot weight and square production were reduced by Meloidogyne incognita only in the susceptible Stoneville 213 cultivar, but not in the resistant McNair 511 cultivar. Joint inoculations with the endophyte and M. incognita revealed that endomycorrhizal development nullified the nematode damage. However, mycorrhizal stimulation of plant development was not affected by root knot nematode activities. Azygospore production was greatest in joint culture on McNair 511 at the high fertility rate and Stoneville 213 at the low fertility level. Nematode egg production/g of root was not affected by G. margarita; however, egg population/plant was increased in Stoneville 213 forming mycorrhizae since the plants produced considerably more susceptible root tissue than did the controls.

ECTOMYCORRHIZAE AND NURSERY PLANTING DATE DO NOT AFFECT INCIDENCE OF LATENT OR NEW FUSIFORM RUST INFECTIONS AFTER FIRST YEAR IN PLANTATIONS

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Seedlings free of visible rust galls from nursery plots sown at 2-week intervals beginning April 15 and either mycorrhizal with Pisolithus tinctorius or with natural symbionts were outplanted in the winter of 1975-76. Survival, growth and number of latent and new fusiform rust infections, were evaluated after one year in the field.

Date sown	Treatment[1]	Percent latent rust[2]	Percent new rust[2]
4/15	NI	0.0[a]	3.2[a]
	Pt	1.0[a]	2.4[a]
4/29	NI	0.0[a]	3.7[a]
	Pt	1.4[a]	1.9[a]
5/13	NI	0.5[a]	3.7[a]
	Pt	0.0[a]	4.0[a]
5/28	NI	0.5[a]	3.3[a]
	Pt	0.9[a]	4.1[a]
6/10	NI	0.4[a]	1.3[a]
	Pt	1.4[a]	1.5[a]
Average	NI	0.3[a]	3.0[a]
	Pt	0.9[a]	2.8[a]

[1] NI = noninoculated seedlings mycorrhizal with natural symbionts; Pt = inoculated seedlings mycorrhizal with Pisolithus tinctorius.

[2] Means followed by a common letter are not significantly different at P=0.05.

Damage by deer browse precluded any meaningful growth data. The data on latent and new rust development shows that the ectomycorrhizal condition of seedlings at planting did not influence their expression of or susceptibility to fusiform rust.

ECTOMYCORRHIZAE AND PLANTING DATE AFFECT RUST INCIDENCE IN FOREST TREE NURSERIES

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Pine seedlings planted on or after May 28 of each year in nurseries need not be sprayed with Ferbam to control fusiform rust. Number of plantable seedlings is markedly reduced by such late plantings. Unless modifications of cultural conditions increase the number of plantable seedlings produced in late plantings, Ferbam sprays are recommended for economical production of pine seedlings. Inoculations with Pisolithus tinctorius did not increase the number of plantable seedlings produced in late plantings.

Date Sown	Treatment[1]	Percent galled[2]	No. plantables[2]	% Mycorrhizae[2] Pt	% Mycorrhizae[2] Total	Production Cost/M Ferbam	Production Cost/M No-spray
4/15	NI	45.7^{b}	27.3^{ab}	0.0^{a}	52.7^{b}	$ 9.00	$ 14.77
	Pt	53.8^{a}	31.4^{a}	23.8^{c}	64.9^{a}	7.85	17.39
4/29	NI	22.4^{c}	26.6^{ab}	0.0^{a}	52.7^{b}	9.23	12.16
	Pt	25.2^{c}	29.6^{a}	11.4^{b}	48.3^{bcd}	8.34	11.26
5/13	NI	22.4^{c}	23.1^{bcd}	0.0^{a}	49.6^{cd}	10.74	14.07
	Pt	21.6^{c}	20.7^{bc}	12.0^{b}	41.2^{cd}	11.89	15.93
5/28	NI	4.1^{d}	18.6^{cde}	0.0^{a}	45.4^{bcd}	13.41	13.96
	Pt	3.5^{d}	17.0^{cde}	14.8^{b}	41.7^{cd}	14.62	15.19
6/10	NI	0.3^{d}	10.2^{e}	0.0^{a}	38.5^{d}	24.26	24.19
	Pt	0.0^{d}	11.1^{de}	24.9^{c}	50.3^{bc}	22.42	22.28
Average	NI	19.0^{a}	21.2^{a}	0.0^{a}	47.8^{a}	13.33	15.83
	Pt	18.8^{a}	22.0^{a}	17.4^{b}	49.3^{a}	13.02	16.41

[1] NI = noninoculated seedlings mycorrhizal with natural symbionts. Pt = inoculated seedlings mycorrhizal with Pisolithus tinctorius.

[2] Means followed by the same letter are not significantly different at P = 0.05.

SUSCEPTIBILITY TO FUSIFORM RUST AND SEEDLING GROWTH INCREASED BY PISOLITHUS TINCTORIUS ECTOMYCORRHIZAE

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ABSTRACT

Three month old, loblolly pine seedlings ectomycorrhizal with Pisolithus tinctorius (Pt) were significantly taller at time of inoculation with Cronartium fusiforme and after one year they had significantly more rust galls than nonmycorrhizal seedling. Susceptibility to fusiform rust was increased in both rust resistant and rust susceptible seedlings and no ectomycorrhizae X host seed source interaction was observed. Either an increase in "target" size or greater succulence of seedlings ectomycorrhizal with Pt caused greater susceptibility of seedlings to infection and gall development by the rust fungus.

Seed source	Mycorrhizal treatment	Seedling height at inoculation[1/]	Percent rust infection[1/]
Rust-Susc.	Non-myc.	41.8^{c}	13.0^{b}
Rust-Resist.	Non-myc.	35.2^{a}	8.0^{a}
Rust-Susc.	Pt	44.4^{d}	24.0^{c}
Rust-Resist.	Pt	40.0^{b}	14.0^{b}
Average	Non-myc.	38.6^{a}	10.5^{a}
	Pt	42.2^{b}	19.0^{b}
Average-Susc.		43.3^{b}	18.0^{b}
Average-Resist.		37.5^{a}	11.0^{a}

[1/] Means followed by a common letter are not significantly different at P = 0.05.

INTERACTION OF TWO VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI AND *PHYTOPHTHORA PARASITICA* ON TWO CITRUS ROOTSTOCKS

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Four-month-old seedlings of two citrus rootstocks, 'Carrizo' citrage (*Citrus sinensis* x *Pancirus trifoliata*) and sour orange (*Citrus aurantium*), colonized or not colonized by mycorrhizae were transplanted to 15-cm plastic pots containing soil infested with *Phytophthora parasitica*. There were nine pots containing three plants for each of 18 treatments. Treatments consisted of two rootstocks ('Carrizo' or sour orange), three mycorrhizae (none, *Gigaspora margarita* or *Glomus macrocarpus*), and three levels of *P. parasitica* (0, 0.7, or 3.5 chlamydospores /g soil) in all possible combinations. Soil temperatures in the greenhouse varied from 22-25 C at night and 26-37 C during the day. After 6 months the seedlings were evaluated for plant response, Phytophthora root rot, and percentage of mycorrhizal fungi in the roots.

In general, the presence of mycorrhizae with or without *P. parasitica* increased shoot weight, stem diameter and root weight on both rootstocks. A higher percentage of roots of 'Carrizo' were colonized by *G. macrocarpus* (41.5%) than *G. margarita* (22.2%) but on sour orange a higher percentage of *G. margarita* (26.7%) occurred than *G. macrocarpus* (20.9%), especially in *P. parasitica* infested soil. Phytophthora root rot ratings were higher on the rootstock 'Carrizo' than they were on the more resistant sour orange rootstock. On 'Carrizo', mean root rot ratings were lower for mycorrhizal than nonmycorrhizal plants.

On sour orange, tap root rot was consistently lower with *G. margarita* and consistently higher with *G. macrocarpus* than with no mycorrhizae. On 'Carrizo' the least amount of tap root loss due to *P. parasitica* was on plants colonized with *G. macrocarpus*, while on sour orange no loss in tap root development occurred on plants colonized with *G. margarita*.

These results indicated that in both growth response and in damage from *P. parasitica*, *G. margarita* was superior on sour orange while *G. macrocarpus* was superior on 'Carrizo'. These results were supported by field tests in which *G. macrocarpus* was also found to predominate on 'Carrizo' seedlings.

MYCORRHIZAL INFECTION OF SOYBEAN ROOTS REDUCES PHYTOPHTHORA ROOT ROT

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Three week old soybean seedling roots infected with Glomus caledonius had twice the fresh weight of non-mycorrhizal roots after both plants were inoculated with Phytophthora megasperma var. sojae Race 1, (PMS-1). Mycorrhizal and non-mycorrhizal plants that were not inoculated with PMS-1 had significantly higher fresh root weights than the plants which were mycorrhizal and inoculated with PMS-1. The results of this experiment indicate that soybean seedling roots infected with Glomus caledonius suffer less damage fromPMS-1 than non-mycorrhizal plants.

EFFECT OF BENOMYL ON SOYBEAN ENDO-MYCORRHIZAE AND THE UPTAKE OF BENOMYL BY MYCORRHIZAL AND NON-MYCORRHIZAL PLANTS

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Soil drenches of benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] at 2.5, 25.0, 125.0, and 250.0 ppm (a.i./dry wt. soil) were added at the time of planting to pots containing "field concentrations" of chlamydospores of the vesicular-arbuscular (VA) mycorrhiza-forming fungus Glomus fasciculatus and soybean seeds. A decrease in mycorrhizal infection as compared to controls occurred when concentrations of 2.5, and 25.0 ppm benomyl were added in one test and when 2.5 ppm was added in another. Higher concentrations of benomyl did not further decrease mycorrhizal infection which had dropped to between 32 and 48% of the root system. Benomyl prevented increased growth due to the VA mycorrhiza at 125.0 ppm or higher in one test and 2.5 ppm or higher in another, even with mycorrhizal infection as high as 48%. Mycorrhizal and non-mycorrhizal soybeans showed similar quantitative patterns of benomyl uptake.

Comparative effects of some organochlorine insecticides - Lindane and Toxaphene on the formation and development of ectotrophic mycorrhiza in seedlings of Picea abies (L.) Karst

by

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ABSTRACT:

Pot experiments were carried out to determine the influence of Lindane and Toxaphene concentrations on the ectotrophic mycorrhizae in seedlings of P. abies.

The doses investigated gave variable results. Evident however was the quantitative reduction in mycorrhizal short roots and intensity of the treated as compared to the control. In general the initiated lateral roots were mostly infected, even in the presence of phytotoxic injuries caused by the organochlorine insecticides.

Effects of chlorinated hydrocarbon insecticides on the formation and development of ectotrophic mycorrhizae in pine seedlings - On mycorrhiza-forming fungi

by

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ABSTRACT:

Laboratory experiments were conducted to study the effects of some chlorinated hydrocarbon insecticides - Lindane and Toxaphene on following mycorrhiza-forming fungi:

Amanita citrina Schaefer

Tricholoma pessundatum (Fr.) Quel. and

Tricholoma saponaceum (Fr.) Quel.

Concentrations investigated were 1, 10, 100, 1000 and 10000 parts per million (ppm), apart from the control.

Though the fungi reacted more or less in different ways to the concentrations of both insecticides, stimulation of mycelial dry substance was recorded through low and sometimes higher doses. Significant reduction of mycelial dry weight was however obtained at 10,000 parts per million.

EFFECT OF A SYSTEMIC FUNGICIDE FOR CONTROL OF FUSIFORM RUST ON GROWTH AND DEVELOPMENT OF MYCORRHIZAL FUNGI

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In laboratory studies, growth of Laccaria laccata, Pisolithus tinctorius, Suillus cothurnatus, S. nirtellus, and S. pinorigidus was measured 1, 2, and 3 weeks after being inoculated onto dishes containing the experimental fungicide, BAS 317 10F, at rates of 0, 1, 3, 5, 10, 40, 80, 100, and 500 µg active ingredients/ml of modified Melins-Norcrans agar. Field treatments were 1) control; 2) Ferbam control; 3) BAS 317 10F, 600 ppm as a soil drench, ½ gal/linear foot of nursery bed, applied once (April 13); 4) same as 3, except concentration was 300 ppm, and applied twice (April 13 & 27); 5) same as 4, but applied 4 times (April 13 & 27, May 11 & 25); and 6) BAS 317 10F, 300 ppm applied as a foliar spray at a rate of 75 gal/acre, four applications, same dates as treatment 5. The fungicide completely inhibited growth of all fungi at all concentrations tested in the laboratory. In August, considerable yellowing and stunting of seedlings was evident in field plots receiving treatments 3, 4, and 5. Roots of 30 seedlings from each plot were examined for mycorrhizae. Results indicated that soil drench treatments severely inhibited mycorrhizal development; some inhibition also was evident on roots of seedlings receiving the foliar spray. By October plants in all plots had developed extensive mycorrhizae. BAS 317 10F either directly or indirectly delays development of mycorrhizal roots on loblolly pine seedlings and that the magnitude of inhibition is dependent on the method of application.

IN VITRO EFFECTS OF SELECTED HERBICIDES ON GROWTH OF MYCORRHIZAL FUNGI

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The herbicides atrazine, bifenox, diphenamid, napropamide, oryzalin, perfluidone, prometryne, and trifluralin were incorporated individually into modified Melin-Norcrans agar at rates of 0, 1, 3, 5, 10, 40, 80, 100, and 500 µg active ingredients/ml and tested for activity against Laccaria laccata, Pisolithus tinctorius, Suillus cothurnatus, S. nirtellus, and S. pinorigidus. Radial growth of each fungus was measured after 1, 2, and 3 weeks. In most cases, the fungi exhibited some degree of growth inhibition at the 3 highest herbicides rates tested; however, these rates were well above what could be expected to occur in the field. At the 3 lowest rates (1, 3, and 5 µg/ml), each fungus was rated as being stimulated, inhibited, or not affected by each of the 8 herbicides. Laccaria laccata was inhibited by trifluralin and bifenox and was not affected by the others. Suillus cothurnatus was inhibited by bifenox, oryzalin, and perfluidone and was not affected by the others. Suillus nirtellus was stimulated by trifluralin and was not affected by the others. Suillus pinorigidus was stimulated by atrazine and bifenox; inhibited by perfluidone, prometryne, and trifluralin; and not affected by the others. Pisolithus tinctorius was stimulated by diphenamid and perfluidone; inhibited by bifenox, napropamide, and oryzalin; and not affected by the others. Results indicate that the herbicides tested may exert selective pressures on certain ectomycorrhizal fungi associated with pine seedlings.

A BRIEF RESUME OF THE STATUS OF MYCORRHIZAE IN ESTABLISHING FOREST PLANTATIONS

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Nearly a half century ago the Research Branch of the U.S. Forest Service, at the Allegheny Forest Experiment Station in cooperation with the Office of Forest Pathology, initiated a study of the mycorrhizal relationships of forest trees. At that early time many forest pathologists believed that mycorrhizae were benign pathogens and not very common. Dr. K. D. Doak and I were therefore assigned the task of determining the frequency and distribution of mycorrhizae on forest trees in the Eastern United States. As we already knew, we confirmed that forest trees were 100 percent mycorrhizal. The project was discontinued when I resigned, hopefully to pursue more meaningful studies at Harvard University and Dr. Doak was transferred to Washington.

In 1936 I published a paper in the Journal of Forestry entitled, "The Role of Mycorrhizae in Afforestation." It reported the first attempt to add pure cultures of ectomycorrhizal fungi to pine seedlings in a natural soil collected in a treeless area in Wyoming, where ectomycorrhizae were absent. I excluded contamination by airborne mycorrhizal fungi from an enclosed growth chamber in a greenhouse by filtering air through cotton; temperature was controlled with a water bath. I did not have electronic filters and air conditioning such as Dr. Marx uses today, but the effects were the same. After two seasons growth, the uninoculated seedlings were yellow and barely alive while the inoculated seedlings were green and normal. Chemical analyses revealed that the mycorrhizal seedlings contained 86 percent more nitrogen, 75 percent more potassium and 234 percent more phosphorous than the nonmycorrhizal seedlings. I recommended in the Journal of Forestry paper that nurseries in the dust-bowl shelter-belt region be started from seed and that pure cultures of ectomycorrhizal fungi be explored to find those which produce the best results in different habitats.

Now, forty years later, the Forest Service at Athens is again pursuing a major study of the utility of mycorrhizae in re- and afforestation. Dr. Marx's spectacular results have stimulated dozens of Federal, State and Private studies in nurseries throughout the world on the possibilities of tailoring seedlings with mycorrhizal fungi to enable them to survive and grow rapidly when transplanted in the field.

The most versatile fungus turned up to date, *Pisolithus tinctorius*, produces mycorrhizae at temperatures as high as forty degrees centigrade. It forms mycorrhizae on strip-mined coal and kaolin wastes at pH's as low as 2.9, and on eroded soils in the Copper Basin of Tennessee. It produces thousands of basidiocarps, each containing billions of spores, in young plantations. It can be added to seed beds either as spores or as fresh or dried mycelial inoculum, and is being produced commercially by Abbott Laboratories and dried to twelve percent moisture by the hundreds of pounds.

This session on the Practical Application of Mycorrhizal Technology will be devoted to a discussion of these present-day revolutionary techniques for tailoring tree seedlings with pure cultures of mycorrhizal fungi.

SUCCESSFUL OVERWINTERING OF PISOLITHUS TINCTORIUS VEGETATIVE INOCULUM AT A VIRGINIA NURSERY AFTER FUMIGATING WITH MC-33

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Successful inoculations in Virginia with Pisolithus tinctorius (Pt) and Thelephora terrestris at the Virginia Division of Forestry Coastal Plain and Mountain nurseries have always followed careful spring fumigation with MC-2 at 1 pound per 100 square feet. At the mountain nursery, however, fumigation with MC-33 and fall seeding are the operational procedures. This study was conducted to determine if successful inoculation could be achieved at the mountain nursery under operational conditions.

Soil fumigation was done during August of 1975 using MC-33 at 6-8 inch depth operationally applied at 285 pounds per acre. The plastic covering was removed after 4 days, and 10-10-10 fertilizer was applied at the rate of 1,200 pounds per acre. The area was then disced, smoothed and bed areas were delineated. The area then remained fallow for approximately 2 months. Bed shaping, fungal inoculation with Pt and seeding with white pine was done in late October.

The study included 4 pairs of inoculated and uninoculated plots separated by buffers of fumigated soil.

One hundred cc. of leached Pt inoculum per square foot was incorporated into each treatment plot. Control plots received sterile peat moss at the same rate. After seeding, operational mulching with rye straw was completed; netting held the mulch in place through the winter months.

Netting and mulch were removed in Spring 1976 so as not to impede seed germination. No fertilizer, herbicides, insecticides or fungicides were applied during 1976. Test plots received operational irrigation, and the small amount of weeding required was done by hand.

Mycelia of Pt were evident in all inoculated test plots in August. The first Pt basidiocarps were found and removed in late September. This is apparently the first successful overwintering of Pt vegetative inoculum in the absence of a host root system. Also, it is the first time the fungus has been established after fumigation with MC-33.

Past failures with mycorrhizal inoculations after MC-33 fumigation may have resulted from a residual effect of the additional chloropicrin in MC-33 (33%) as opposed to the amount in MC-2 (2%). In this study, the 2-month delay between fumigation and seeding afforded adequate time to eliminate the residual toxicity of chloropicrin. While this extended delay may be impractical for spring fumigation and seeding, it should pose no problems at nurseries where crop establishment is a fall operation.

Inoculation of one and two year-old Scotch pine seedlings with *Pisolithus tinctorius*

Jerry Grebasch, Roy Hatcher, and Robert Anderson[1/]

One and two-year old Scotch pine seedlings were inoculated with *Pisolithus tinctorius* vegetative inoculum at the Iowa State Nursery during May, 1976. Two six-foot seedling plots were inoculated for each age class by digging a small trench about three to four inches deep and six feet long between the two center rows, placing one liter of *P. tinctorius* into the trench and covering it with the soil that was removed from the trench. The control plots did not receive any treatment.

The seedlings were lifted and examined after the growing season in September, 1976. *Pisolithus tinctorius* had colonized all of the seedlings in the two rows adjacent to the trench. The percentage of roots on each seedling infected by *P. tinctorius* ranged from 20 to 70%. The fungus had moved into the adjacent rows but comprised about 10-20% of the total ectomycorrhizae percentage. *Pisolithus tinctorius* was not found in the third row from the trench.

No appreciable differences were noted in the percentage of roots classified as ectomycorrhizae in treated and control plots for both age classes. The seedlings average from 60 to 67%. The treated one-year old seedlings were 10 mm larger than the controls and had a 32% increase in shoot weight. The two-year old treated seedlings were the same height as the controls but the controls were 18% larger in this treatment.

CONCLUSIONS: (1) *Pisolithus tinctorius* vegetative inoculum can be used to inoculate one and two-year old nursery seedlings. (2) A Georgia strain of *Pisolithus tinctorius* (provided by Don Marx) colonized Scotch pine in Iowa. (3) The fungus provided effective colonization of the root systems only in the rows adjacent to the point of inoculation. (4) The percent of root systems classified as ectomycorrhizae did not increase through inoculation but the naturally occurring ectomycorrhizal fungi were replaced by *P. tinctorius* in the treatment plots.

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COMPARISON OF ABBOTT'S AND ATHENS' DRIED INOCULUM OF PISOLITHUS TINCTORIUS AT DIFFERENT DENSITIES WITH GOOD OR POOR SOIL FUMIGATED FOR ECTOMYCORRHIZAL DEVELOPMENT ON LOBLOLLY PINE AND NORTHERN RED OAK SEEDLINGS IN MICROPLOTS AT WHITEHALL

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This study will be conducted at the Whitehall Experiment Forest Nursery, Athens, Georgia. Microplots containing properly and improperly fumigated soil will be artificially infested with different rates of inoculum of Pisolithus tinctorius produced by Abbott Laboratories and the inoculum produced at the Institute for Mycorrhizal Research and Development. The objective of this study is to determine if the inoculum commercially produced by Abbott is as good for ectomycorrhizal synthesis on seedling roots as the inoculum produced at the Athens facility. Another objective will be to ascertain the value of soil fumigation in the establishment of P. tinctorius on loblolly pine (Pinus taeda L.) and northern red oak (Quercus rubra) seedlings.

Mid-year evaluation data should be available for presentation at the Mycorrhizal Conference.

ECTOMYCORRHIZAL DEVELOPMENT BY DIFFERENT ISOLATES AND INOCULUM RATES OF PISOLITHUS TINCTORIUS ON NORTHERN RED OAK SEEDLING

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Freshly collected isolates of Pisolithus tinctorius (Pt) from pin oak (isolate 136), sawtooth oak (isolate 145), white oak (isolate 177), and loblolly pine (isolate 138) were each used at two inoculum rates (150 and 300 cc/sq ft) to infest fumigated soil in microplots in an experimental nursery near Athens, Georgia. Acorns of northern red oak were planted in April 1976 and seedlings were lifted and evaluated in December 1976. Seedlings received 250 lbs/acre of 10-10-10 and 900 lbs/acre of lime at planting and were side dressed twice with 50 lbs/acre NH_4NO_3 during the growing season. Results are as follows:

Isolate	Inoc. Rate	Height (cm)	Stem dia(mm)	Fresh weights (gm) Top	Roots	Total	% Ectomycorrhizae by Pt	Other	Total
Control[1/]	0	23.3	6.6	4.3	20.8	25.1	0	28	28
138	150	29.6*	7.6*	7.3*	25.8*	33.1*	9*	23	32*
	300	32.0*	7.8*	7.2*	26.7*	33.9*	17*	20	37*
136	150	25.1	7.0	4.5	22.4	26.9	2	21	23
	300	28.1	7.4	5.9	25.4	31.3	3	23	26
145	150	24.5	7.1	5.0	22.1	27.1	1	21	22
	300	23.2	7.1	4.6	22.5	27.1	1	23	24
177	150	24.5	7.0	4.4	21.2	25.6	1	23	24
	300	27.2	7.4	5.8	24.3	30.1	1	27	28

[1/] 300 ml/sq ft of an equal mixture of autoclaved inoculum of all four isolates.

* denotes significant differences at P =0.05.

The results from this test, as well as others, show that there is significant variation in the ability of certain isolated of P. tinctorius to form ectomycorrhizae on northern red oak. Only the pine isolate (#138) formed sufficient ectomycorrhizae to significantly stimulate seedling growth. It is not known why the oak isolates did not form as many ectomycorrhizae as did the pine isolate. Similar results were obtained with these same isolates tested on loblolly pine seedlings in the growth room. The pine isolate (#138) was always superior. There is no difference in growth of these isolates on various agar media at different temperatures.

We observed in this study that Thelephora terrestris forms ectomycorrhizae with northern red oak. Also we found that inoculum of Pisolithus must be incorporated in soil deeper than the normal 4 to 6 inches if species, such as northern red oak, with deep rooting habit are to be evaluated. At least half of the lateral roots of the oak seedling in this study developed below the zone of soil infestation and, therefore, they did not have a good opportunity to become infected by P. tinctorius.

THE EFFECTS OF DIFFERENT RATES OF VEGETATIVE INOCULUM OF PISOLITHUS TINCTORIUS ON ECTOMYCORRHIZAL DEVELOPMENT AND GROWTH OF LONGLEAF AND SHORTLEAF PINES.

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Five rates of non-dried vegetative inocula of Pisolithus were added to fumigated soil at the Ashe Nursery, Mississippi in April 1976. Approximately 26,000 longleaf pine and 26,000 shortleaf pine seedlings were lifted and evaluated in December 1976. Results are as follows:

Treatment	Pine host	Fresh weight of seedlings (gm)	% Ectomycorrhizae Pisolithus	% Ectomycorrhizae Total	Percent culls
Control	longleaf	12.8	3[1/]	23	23
	shortleaf	14.6	3[1/]	61	33
2.7 l/m^2 (250 cc/ft^2)	longleaf	17.8	25	43	17
	shortleaf	17.8	24	60	24
2.16 l/m^2 (200 cc/ft^2)	longleaf	19.9	26	40	19
	shortleaf	21.9	32	62	24
1.62 l/m^2 (150 cc/ft^2)	longleaf	22.2	20	35	20
	shortleaf	19.1	31	60	24
1.08 l/m^2 (100 cc/ft^2)	longleaf	16.7	19	34	14
	shortleaf	19.1	37	64	28
.54 l/m^2 (50 cc/ft^2)	longleaf	16.9	17	35	16
	shortleaf	14.8	19	61	24

[1/] Natural occurrence of Pisolithus in nursery.

The results showed that growth and quality of longleaf and shortleaf pine seedlings are increased by Pisolithus ectomycorrhizae and also, that an inoculum rate of 1.08 l/m^2 of soil surface is as effective for ectomycorrhizal development as are greater or lesser rates.

THE INFLUENCE OF INOCULUM RATE ON THE EFFECTIVENESS OF STANDARD (NONDRIED) AND DRIED VEGETATIVE MYCELIAL INOCULUM OF PISOLITHUS TINCTORIUS FOR ECTOMYCORRHIZAL DEVELOPMENT ON LOBLOLLY PINE.

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Standard leached inoculum of P. tinctorius in vermiculite is very heavy and, therefore, difficult to transport. The purpose of this test was to determine whether the standard inoculum could be dried (12% moisture) at 30° C for 56 hrs and still function as ectomycorrhizal inoculum. Drying reduced weight of inoculum by 3.5 times. Leaching, the use of pressure to remove excess water after leaching, and drying reduces inoculum volume by half. Inoculum was incorporated 4 to 6" deep in fumigated soil and planted (April 1976) with loblolly pine seed in microplots (4.5 sq ft) at the Athens nursery. Seedlings (a total of 7,900) were lifted and evaluated in December 1976. The results are as follows:

Inoculum source	cc of inoculum per sq ft soil surface	Percent ectomycorrhizae formed by: Pisolithus	Other fungi	Total
Standard (nondried)	200	38	29	67
	100	30	44	74
	50	30	47	77
	25	16	44	60
Dried (12% moisture)	200	53	24	77
	100	34	42	76
	50	40	36	76
	25	12	52	64
Control	0	0	61	61

The results show clearly that dried inoculum is as good as, if not better than, standard inoculum for development of Pisolithus ectomycorrhizae. The dried inoculum is more evenly mixed into the soil and, thereby, may be more effective. It appears also that 100 cc of inoculum/ft^2 of soil surface may be a practical inoculum rate to use for adequate ectomycorrhizal development by Pisolithus tinctorius.

A PRELIMINARY TEST OF PISOLITHUS TINCTORIUS ON PECAN

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Observations by Menge in California and other in the Southwest suggest that P. tinctorius is an ectomycorrhizal fungus of pecan. A small test was initiated in April 1976 in microplots with the Curtis variety of pecan and a pine isolate (#138) of P. tinctorius (Pt) in fumigated soil. Seedlings received 250 lb/acre of 10-10-10 and 900 lb/acre of lime at planting and were side dressed twice with 50 lb/acre NH_4NO_3 during the growing season. Seedlings were lifted and evaluated in December 1976. The results are as follows:

Treatment	Height (cm)	Stem dia(mm)	Fresh weights (gm) Top	Roots	Total	% Ectomycorrhizae by: Pt	Other	Total
Control (killed inoculum)	28.9	6.4	3.9	18.0	21.9	0	56	56
Pisolithus (300 ml/ sq ft)	32.8*	7.1*	5.2*	22.0*	27.2*	24*	35*	59

* denotes significant differences at P = 0.05

Pisolithus formed ectomycorrhizae on and improved significantly the growth of pecan in this study. The morphology of the ectomycorrhizae were similar to those formed on northern red oak reported in another abstract. Pecan is similar to northern red oak in its deep rooting habit. Unfortunately, inoculum of P. tinctorius was only incorporated 4-6" deep in soil and therefore was not located in the major root zone for maximum ectomycorrhizal development. A greater incidence of Pisolithus ectomycorrhizae may have stimulated more growth. Thelephora terrestris formed ectomycorrhiza on pecan seedlings and formed basidiocarps epiphytically on seedling stems.

STORAGE POTENTIAL OF STANDARD AND DRIED VEGETATIVE MYCELIAL INOCULUM OF PISOLITHUS TINCTORIUS.

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The ability of vegetative mycelial inoculum of P. tinctorius to survive in storage is essential to its practical application in forestry. Mass inoculum of P. tinctorius was grown in 1.5 l volumes of vermiculite-peat moss-nutrient at room temperature for 4 months. After leaching by conventional procedures, half the volume was dried (30° C for 56 hrs) to 12% moisture. Standard (nondried) and dried inoculum was stored in 15 ml volumes in test tubes at different temperatures. At two week intervals, inoculum was used to infest soil to test its ability to form ectomycorrhizae on loblolly pine seedlings in 4 months in the Athens growth room. The results are:

Inoculum source	Storage temperature	Weeks of storage 0	1	3	5	7	9	11	13	15
Standard	5° C	57	48	41	47	43	41	17	6	1
	23° C	57	55	35	38	38	44	12	4	0
	30° C	57	42	33	38	28	14	0	1	0
Dried (12% moisture)	5° C	50	31	37	40	42	36	9	3	1
	23° C	50	44	41	36	42	21	3	2	0
	30° C	50	48	48	37	30	18	0	0	0

The results indicate that following an initial reduction in inoculum viable during the first week of storage, Pisolithus in vermiculite-peat moss is viable for 7 - 9 weeks storage at 5° or 23° C and 5 to 7 weeks storage at 30° C. Storage in larger volume, such as 4-5 ft^3 volumes, should increase storage potential.

GROWTH AND ECTOMYCORRHIZAL DEVELOPMENT OF LOBLOLLY PINE SEEDLINGS IN NURSERY SOIL INFESTED WITH PISOLITHUS TINCTORIUS AND THELEPHORA TERRESTRIS IN VIRGINIA

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Vegetative mycelial inocula of Pisolithus tinctorius and Thelephora terrestris were artificially introduced into fumigated soils of two state nurseries in Virginia in early 1975. After one growing season, seedlings with Pisolithus and Thelephora ectomycorrhizae had 57 and 31 percent greater fresh weights in the coastal plain nursery and 40 and 20 percent greater weights in the mountain nursery respectively, than the control seedlings. Soil infestation with both fungi increased ectomycorrhizal development by about 50 percent in both nurseries. Half normal (34 kg/ha) or normal nitrogen (68 kg/ha NH_4NO_3) fertilizer rates applied at the coastal plain nursery and no added nitrogen fertilizer at the mountain nursery did not affect seedling growth regardless of fungus treatment. The implications of these results are discussed.

EVALUATION OF PISOLITHUS TINCTORIUS INOCULUM PRODUCED BY ABBOTT LABORATORIES FOR ECTOMYCORRHIZAL DEVELOPMENT ON VARIOUS PINE SPECIES IN SIXTEEN TREE NURSERIES IN THE SOUTHERN HALF OF THE UNITED STATES

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This study represents the first test of commercially produced (Abbott Laboratories) inoculum of an ectomycorrhizal fungus. Different rates (150, 100, and 50 cc/sq ft) of Abbott inoculum and one rate (100 cc/sq ft) of Athens-grown inoculum will be tested in 18 experiments in 16 private, state or federal nurseries in 15 states of the southeast, south and southwest on seven pine species. The purpose is to determine the effectiveness of the Abbott inoculum for ectomycorrhizal development by Pisolithus tinctorius and the effects on seedling quality. This is a cooperative research venture between the USDA Forest Service, Abbott Laboratories, 7 state forestry agencies, 7 forest industries and Regions 3, 5, and 8 of the National Forest System.

Mid-year evaluation data should be available for presentation at the Mycorrhizal Conference.

PRODUCTION LEVEL DEMONSTRATION OF THE EFFICACY OF PISOLITHUS TINCTORIUS INOCULUM PRODUCED BY ABBOTT LABORATORIES FOR GROWTH AND ECTOMYCORRHIZAL DEVELOPMENT ON PINE IN THREE NURSERIES

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This study will determine the feasibility of commercial application of vermiculite-based mycelial inoculum of Pisolithus tinctorius, produced by Abbott Laboratories for the production of pine seedlings in nurseries. Nurseries in Oklahoma (Weyerhaeuser), Florida (Buckeye Cellulose), and Mississippi (Region 8) will each have five 200 linear feet of nursery beds infested with mycelial inoculum and planted to loblolly, slash, or shortleaf pine, respectively. Following discrete sampling at the end of one growing season, all seedlings will be lifted by conventional nursery procedures, graded and counted. Resulting data will ascertain the effects of Pisolithus ectomycorrhizal development from the Abbott inoculum on pine seedling quality.

Mid-year evaluation data should be available for presentation at the Mycorrhizal Conference.

USE OF BASIDIOSPORES OF PISOLITHUS TINCTORIUS FOR LARGE-SCALE PRODUCTION OF "TAILOR-MADE" LOBLOLLY PINE SEEDLINGS AT FORT TOWSON NURSERY IN OKLAHOMA

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In 1976, several methods were used to infest fumigated soil with basidiospores of Pisolithus at Weyerhaeusers nursery in Fort Towson, Oklahoma. The purpose was to find a treatment which would infest soil evenly and produce significant quantities of Pisolithus ectomycorrhizae on loblolly pine seedlings and improve their quality. Five treatments were tested; each treatment had 5 replicates of 100 linear feet (400 sq ft/replicate) of nursery bed each. The treatments were 1) spores mixed in water and wood fiber (hydromulch) and applied immediately after seeding; 2) spores dusted onto soil surface after seeding but before hydromulching; 3) spores injected into soil with tractor-mounted drills immediately before seeding and hydromulching; 4) spores dusted onto soil and seedlings six weeks after seeding; 5) spores in water suspension (wetting agent added) applied to soil and seedlings by tractor 6 weeks after seeding; and 6) NO treatment. Spores in all treatments were applied at a rate of 50 mg spores/ft^2 (540 mg/m^2) of soil surface.

In January 1977, the seedlings (a total of approximately 355,000) were lifted by machine, graded, counted and bagged by nursery personnel. Seedlings in strip plots (10% sample) were removed prior to machine-lifting and evaluated in detail.

The results showed that treatments 1 and 2 reduced the number of cull-grade seedlings by approximately 30%. Treatment 3 reduced culls by approximately 18%. The other treatments did not appreciably influence the cull percentage. Reduction in the number of culls was positively related to the number of seedlings with Pisolithus ectomycorrhizae and the percentage of short roots infected by Pisolithus. Based on these results it appears that badisiospore of Pisolithus can be used on an operational basis to assure ectomycorrhizal development and improve nursery production of loblolly pine seedlings.

MYCORRHIZAL INOCULATION AND GROWTH OF PINE SEEDLINGS ON ERODED SOIL*

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ABSTRACT

Mycorrhizal fungi were grown in 2 liter jars and nursery plots filled with a mixture of sand and soil were infested with 2 jars of each symbiont test. Pine seedlings were grown for 30 weeks and removed from the soil and growth measured. Only 2 of the 8 pine species tested showed, under these adverse conditions, a good response to mycorrhizal inoculation: Pinus pseudostrobus had a growth increase of 164%, 115%, and 57% when inoculated with Lepiota lutea, Pisolithus tinctorius, and Laccaria laccata respectively in comparison to uninoculated control seedlings. Pinus radiata showed a growth increase of 125% with L. Lutea, 78% with P. tinctorius and 62% with L. laccata.

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EFFECTS OF PISOLITHUS TINCTORIUS ECTOMYCORRHIZAE ON GROWTH OF LOBLOLLY AND SHORTLEAF PINES ON PLOTS AMENDED WITH SEWAGE SLUDGE IN THE TENNESSEE COPPER BASIN

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Loblolly and shortleaf pine seedlings were tailored in nursery beds with the ectomycorrhizal fungus, Pisolithus tinctorius (Pt). Control seedlings were allowed to become ectomycorrhizal with Thelephora terrestris which occurs naturally and abundantly in the southeast. Seedlings were planted on a severely eroded site in the Tennessee Copper Basin in plots amended with dried sewage sludge (34 metric tons/ha) or fertilizer (10-10-10 at 896 kg/ha) and lime (986 kg/ha Ca). After two years, shortleaf pine appears to be unsuited for planting on the severe sites in the Copper Basin and grew poorly compared to loblolly pine. None of the treatments were statistically superior for growth of shortleaf pine. Loblolly pine, however, responded positively to both sludge and Pt ectomycorrhizae. Loblolly pine seedlings with Pt ectomycorrhizae produced 87% more volume (D^2H) than noninoculated seedlings on plots that had been amended with sewage sludge. There was no mycorrhizal effect on plots amended with fertilizer and lime. Sewage sludge stimulated 70% more volume growth of seedlings with Pt ectomycorrhizae than fertilizer and lime; sludge did not stimulate control loblolly pine seedlings.

EFFECTS OF PISOLITHUS TINCTORIUS ECTOMYCORRHIZAE ON GROWTH OF LOBLOLLY AND VIRGINIA PINES IN THE TENNESSEE COPPER BASIN

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Loblolly and Virginia pine seedlings were tailored in a tree nursery with the ectomycorrhizal fungus, Pisolithus tinctorius. Control seedlings were treated identically except that they were allowed to become ectomycorrhizal by Thelephora terrestris which occurs naturally. Identical experiments were installed at two severely eroded sites in the Copper Basin of Tennessee. After two years, there was no difference in height, root collar diameter or volume growth between the two pine species. Seedlings of both tree species with Pisolithus ectomycorrhizae, however consistently grew better than seedlings with Thelephora terrestris ectomycorrhizae based on height, stem collar diameter and seedling volume measurements. The growth differences between treatments were significant at one location, but statistical significance was precluded at the other location by excessive variation among replicates of control seedling with T. terrestris ectomycorrhizae.

GROWTH OF LOBLOLLY AND SHORTLEAF PINE SEEDLINGS AFTER TWO YEARS ON A STRIP-MINED COAL SPOIL IN KENTUCKY IS STIMULATED BY PISOLITHUS ECTOMYCORRHIZAE AND "STARTER" FERTILIZER PELLETS

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Pine seedlings "tailored" in a nursery with either Pisolithus tinctorius (Pt) or natural (NI) ectomycorrhizae were outplanted on a pH 3.6-4.3 coal spoil in Kentucky in March 1975; a 21 gram "starter" fertilizer pellet (20-10-5 formulation) was placed in the closing hole (7.5 cm from roots) of half the seedlings. Seedlings were measured and foliar analysis were made at the end of the second growing season. The results are as follows:

Treatment	Percent survival	Height (cm)	Stem dia(cm)	PVI(X 10^2)[1/]	Needles %N	Needles %P
			Loblolly			
Pt alone	84	95	2.5	128	1.5	.11
NI alone	87	55	1.3	98	1.2	.13
Pt + pellet	88	107	2.7	196	1.2	.11
NI + pellet	80	84	1.8	57	1.0	.10
			Shortleaf			
Pt alone	66	55	1.6	36	1.7	.11
NI alone	63	41	1.2	9	1.4	.12
Pt + pellet	69	79	2.4	84	1.5	.11
NI + pellet	70	62	1.8	53	1.3	.12

[1/] PVI = Plot Volume Index (stem dia^2 X height X No. surviving trees).

The results are obvious in regard to ectomycorrhizae. However, seedlings with the "starter" pellet are chlorotic and nearly nitrogen deficient after the second year because of lack of abundant, readily available N which was present during the first year.

SURVIVAL AND GROWTH OF PINE SEEDLINGS WITH PISOLITHUS ECTOMYCORRHIZAE AFTER TWO YEARS ON REFORESTATION SITES IN NORTH CAROLINA AND FLORIDA

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Two-year field data indicated that Pisolithus ectomycorrhizae increases survival and growth of pine seedlings on routine reforestation sites. The seedlings were "tailored" in nurseries with Pisolithus ectomycorrhizae. Control seedlings had ectomycorrhizae formed by naturally occurring fungi in the nurseries. Loblolly pine on a poor site and Virginia pine on good and poor sites in North Carolina; slash pine on two good sites and sand pine on a good and poor site in Florida had better survival with Pisolithus ectomycorrhizae than with natural ectomycorrhizae. Significant increases in plot volumes due to Pisolithus ectomycorrhizae over natural ectomycorrhizae were observed; they varied from 25% for loblolly pine in North Carolina to 450% for sand pine in Florida and white pine in North Carolina. Pisolithus persisted on roots and reproduced well on seedlings of all pine species especially on poor sites. Indigenous symbiotic fungi also formed significant quantities of ectomycorrhizae particularly on the better sites (submitted to Forest Science).

ABSTRACT

GROWTH AND ECTOMYCORRHIZAL DEVELOPMENT OF LOBLOLLY PINE SEEDLINGS IN FUMIGATED AND NON-FUMIGATED NURSERY SOIL INFESTED WITH DIFFERENT FUNGAL SYMBIONTS IN OKLAHOMA

Marx, D.[1], W. Morris[2], and J. Mexal[2]

Presented by W. Morris

A study involving vegetative mycelial inoculum of *Cenococcum graniforme* (Cg), *Thelephora terrestris* (Tt), and *Pisolithus tinctorius* (Pt) and three densities of Pt basidiospores (108, 324, 648 mg/m^2) was conducted at Ft. Towson Forest Regeneration Center near Ft. Towson, Oklahoma. These ectomycorrhizal fungi were introduced into methyl bromide-fumigated or non-fumigated plots. The vegetative mycelial inoculum and the spores were incorporated into the soil before sowing. Seedlings were grown as normal operational seedlings.

In December the seedlings were analyzed for percent plantable seedlings, height, stem diameter, shoot and root fresh weights and percent mycorrhizal infection. The results indicate that within the fumigated plots the vegetative mycelial inoculum of Cg, Tt, Pt and the Pt basidiospores at three densities increased the percent plantable seedlings 77%, 155%, 140%, 81%, 79%, and 75%, respectively, over the control. Within the non-fumigated plots, the differences were not as large, with the Tt and Pt vegetative mycelium having the greatest effects.

All treatments within the fumigated plots increased seedling height and shoot fresh weights. Stem diameter was increased by all treatments except vegetative mycelial inoculum of Cg. Root fresh weight was increased by the vegetative mycelial inoculum of Tt and Pt. In the non-fumigated plots, none of the treatments increased seedling height, shoot fresh weight, or root fresh weight. Stem diameter was increased by Pt vegetative mycelial inoculum and the two higher levels of Pt spores.

Vegetative mycelial inocula of Pt were superior to spores as a media for Pt infection of roots in both fumigated and non-fumigated soils.

This research indicates that inoculation with mycorrhizal fungi can greatly increase the percent of plantable seedlings when the endogenous inoculum levels have been reduced by fumigation. Seedling growth was also affected by mycorrhizal inoculation within the fumigated soil. Mycorrhizal inoculation in non-fumigated soil had a lesser effect than in fumigated soil with Pt and Tt vegetative mycelial inoculum having the most effect.

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Time Required: 15 min.

TITLE: Mycorrhizal treatment related to better survival and growth for outplanted loblolly pine in Arkansas and Oklahoma.

AUTHORS: Mexal, J.[1], D. Marx[2], and W. Morris[1]

PRESENTED BY: J. Mexal

Loblolly pine (P. taeda L.) seedlings artifically inoculated with Pisolithus tinctorius or Thelephora terrestris as well as seedlings with naturally occurring mycorrhizae were outplanted on eight sites in Arkansas and Oklahoma in a randomized complete block design. Seedlings had either: (1) high incidence of Pisolithus mycorrhizae, (2) low incidence of Pisolithus mycorrhizae, (3) no Pisolithus mycorrhizae, but a high incidence of Thelephora, (4) high incidence of natural inoculum, or (5) low incidence of natural inoculum. Treatments 4 and 5 were associated with high and low levels of ammonium sulfate fertilization in the nursery, respectively.

Ranks for the above treatments were 1>4>5>2>3 measured by plot volume index (height x caliper^2 x survival) after one year in the field. Survival for seedlings with a high incidence of Pisolithus or natural mycorrhizae was 16% and 12%, greater than treatment 3, respectively. Plot volume index was increased 54% and 43%, respectively.

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Time required: 10 min.

SURVIVAL AND GROWTH INCREMENT OF LOBLOLLY PINE SEEDLINGS OUTPLANTED WITH DIFFERENT MYCORRHIZAL ASSOCIATIONS ON KAOLIN SPOIL

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Loblolly pine seedlings were grown and "tailored" with ectomycorrhizae formed by Pisolithus tinctorius (Pt), Cenococcum graniforme (Cg) and Thelephora terrestris (Tt) in an electronically air filtered growth room. These seedlings were planted in a split-plot experimental design on two recently mined and graded kaolin spoil sites near Deepstep, Georgia. Site II did not have a Thelephora mycorrhizal treatment. One half of all plots was fertilized with 85 g of 10-10-10 fertilizer placed in each of two slits 10 cm from each seedling. Measurements of height and stem diameter were taken shortly after planting. After one growing season, height, stem diameter and percent survival were determined and incorporated in a plot volume index (PVI) increment (height X (stem diameter)^2 X number of surviving trees in a plot). This data is summarized below.

Treatment	Height increment[1] (cm)	Stem diameter increment[1] (mm)	Percent survival	PVI increment[1]
		Site I		
Pt nonfertilized	6.98 a	2.65 a	92	1078 a
Pt fertilized	6.55 a	2.25 ab	74	592 b
Cg nonfertilized	5.63 ab	1.63 bc	84	300 b
Cg fertilized	7.38 a	2.05 abc	54	403 b
Tt nonfertilized	4.13 b	1.17 b	74	100 b
Tt fertilized	3.35 b	1.80 abc	62	162 b
		Site II		
Pt nonfertilized	13.93 a	5.90 a	87	10183 a
Pt fertilized	23.42 b	6.33 a	85	19086 a
Cg nonfertilized	12.37 a	6.63 a	92	11962 a
Cg fertilized	21.70 b	6.30 a	71	14642 a

[1] Means sharing the same letter are not significantly different at the 5% level.

The nonfertilized Pisolithus treatment on Site I had greater survival and growth increment over all the other treatments. Fertilization tended to decrease seedling survival, on both sites, regardless of mycorrhizal conditions.

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MYCORRHIZAL INVESTIGATIONS IN THE GREAT PLAINS

In April 1975, in a cooperative study with Dr. Richard Tinus, vegetative mycelium of each of six ectomycorrhizal fungi and pine duff were mixed separately with container potting medium (1:1 vermiculite-peat moss) at ratios of 10 and 20% by volume, immediately seeded with *Pinus ponderosa* or *P. sylvestris*, and seedlings grown in the U.S.F.S. greenhouse at Bottineau, North Dakota for one year. Good ectomycorrhizal development occurred on seedlings with *Pisolithus tinctorius* or pine duff. For *P. sylvestris*, treatment with duff resulted in heavier fresh stem weights and larger root-collar diameters than treatment with *P. tinctorius*, but not with controls (no inoculation). For *P. ponderosa*, duff and *P. tinctorius* treatments did not improve seedling growth over that of non inoculated seedlings. Inoculation with a mixture of six mycorrhizal fungi resulted in larger stem weights, root-collar diameters, and shoot/root ratios than with no inoculation. Mixing mycorrhizal inoculum in potting medium at 20% was no better than using a 10% rate. Preliminary results after the first season in an outplanting with soil pH 8.4 indicate very good survival and growth of both tree species from pine duff treatment.

In April, 1977, tests with commercially produced *Pisolithus tinctorius* mycorrhizal inoculum will be installed at the newly established U.S.F.S. nursery at Albuquerque, N.M., and also at Bottineau, N.D. Objectives of the tests will be to compare (1) inoculum produced in large fermentors in less than one month with inoculum produced in the laboratory in 3 to 4 months, and (2) growth and survival of seedlings in the nursery and in outplanting sites. *P. ponderosa* will be planted at Albuquerque; *P. ponderosa* and *P. sylvestris* will be planted in containers at Bottineau. Seedlings will be grown for one year and outplanted.

A test with three fumigants (methyl bromide-MB, 98% at 46 and 64 kg/ha, and 67% at 46 and 89 kg/ha; mylone, 50%, 3,5-dimethyltetra hydro- 1,3,5, 2H-thiadiazine-2-thione at 28 and 37 kg/ha; vorlex, 20% methyl isothiocyanate and 80% chlorinated C_3 hydro-carbons at 38 and 61 l/ha) and one fungicide (terraclor, 75% pentachloronitrobenzene at 20 kg/ha) was conducted on Mandan silt loam to determine effects on emergence, damping-off, survival, and growth of *Lonicera tatarica, Elaeagnus angustifolia, Shepherdia argentea,* and *Fraxinus pennsylvanica* seedlings, and on development of endomycorrhizae. Seedbeds were treated in August and seeded in October 1974. Seedling growth in MB plots (heights, 98% at 46 and 64 kg/ha; 67% at 89 kg/ha; weights, 98% at 46 kg/ha; diameters, 98% at 46 and 64 kg/ha) after one year was lower than in control plots. Percent and density of endomycorrhizal development were greater for each species in nonfumigated than in methyl bromide fumigated seedbeds.

INOCULATION OF CONTAINER GROWN PINES WITH BASIDIOSPORES OF PISOLITHUS TINCTORIUS

John L. Ruehle

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A greenhouse study to determine the best procedure for inoculating Pinus taeda grown in styroblock containers with various quantities of basidiospores of Pisolithus tinctorius (Pt) was conducted. Styroblock-8 units were filled with a peat-vermiculite mixture 1:1 v/v and seeded with stratified P. taeda seed. Container units were placed in a special chamber immediately after seeding and dusted with the following quantities of screened Pt basidiospores: 50 mg/ft^2, 100 mg/ft^2 , 200 mg/ft^2. After each unit was dusted, a mist spray of tap water was applied for 2 minutes to rinse the spores into the medium in each of the 80 cavities in each unit. Basidiospores were applied at time of seeding and at four week intervals thereafter up to 12 weeks. Each spore treatment was replicated 5 times. Eighteen weeks after seeding, 10 seedlings were removed at random from each unit and evaluated for mycorrhizal development and seedling growth.

The greatest Pisolithus ectomycorrhizal development averaged 25% of the short roots and occurred in the seedling group that had been dusted 4 weeks after seeding. Seedlings were most receptive to mycorrhizal synthesis immediately after germination (4th week after seeding). At later periods, contamination from airborne Thelephora terrestris created competition for Pt and probably accounted for poor synthesis at the 8- and 12-week periods. Spore treatment and time of application had no effect on seedling growth. There was no difference in total ecto-mycorrhizal development among spore treatments at each of the time intervals.

DEVELOPING ECTOMYCORRHIZAE ON CONTAINERIZED PINE SEEDLINGS

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ABSTRACT

Effects of fertility, growing medium and artificial soil infestation with *Pisolithus tinctorius* on ectomycorrhizal development on container grown, loblolly pine seedlings were tested in the greenhouse. These preliminary tests showed that artificial infestation with *P. tinctorius* will produce satisfactory ectomycorrhizal development with the right combination of fertility and growing medium. Milled pine bark is a promising and inexpensive alternative to the costly peat-vermiculite growing medium currently used in containerization programs in the Southeastern United States.

EVALUATION OF PISOLITHUS TINCTORIUS INOCULUM PRODUCED BY ABBOTT LABORATORIES FOR ECTOMYCORRHIZAL DEVELOPMENT ON VARIOUS TREE SPECIES GROWN IN CONTAINERS.

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ABSTRACT

A feasible means of forming ectomycorrhizae on containerized conifer seedlings prior to outplanting would improve survival and early growth following outplanting, particularly on stressed or adverse sites. Vegetative inoculum of Pisolithus tinctorius produced by Abbott Laboratories shows promise as a commercial product for inoculating containerized conifers. This cooperative study is designed to test the efficacy of the Abbott inoculum to synthesize ectomycorrhizae on ten economically important conifers grown in containers. Two different species will be studied at each of five locations during 1977 as follows: Pinus clause and P. elliottii - Athens, Georgia; Pinus echinata and P. palustris - Pineville, Louisiana; Pinus taeda and P. virginiana - Clayton, North Carolina; Pseudotsuga menziesii and Tsuga heterophylla - Corvallis, Oregon; and Pinus ponderosa and P. sylvestris - Bottineau, North Dakota.

Mid-year evaluation data should be available for presentation at the Mycorrhizal Conference.

EFFECT OF ARTIFICIAL INOCULATION OF FUMIGATED NURSERY BEDS WITH SOIL CONTAINING ENDOMYCORRHIZAL FUNGI ON GROWTH OF SWEETGUM AND SYCAMORE SEEDLINGS

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Abstract

Research was conducted while the author was a graduate student at North Carolina State University, Raleigh, North Carolina.

Experiments were conducted on sweetgum (Liquidambar styraciflua L.) and sycamore (Platanus occidentalis L.) at six nurseries throughout the southeast. Seedbeds at five nurseries were treated with methyl bromide at rates ranging from 300 to 450 pounds per acre. At one nursery, telone-C at 40 gallons per acre was used to fumigate the soil. Six treatments involving different rates and combinations of N, P, and K were split with half of the plots receiving an application of soil containing endomycorrhizal spores, root fragments, and litter from the upper 4 inches of a hardwood stand. The addition of the forest soil inoculum increased production of large diameter sweetgum seedlings at three of the five nurseries that were fumigated with methyl bromide. No effect on large diameter sycamore seedlings was observed. The inoculum had no effect on sweetgum or sycamore at the nursery treated with telone-C. An interaction between inoculation and phosphorus application was observed at one nursery where methyl bromide had been used. With no addition of phosphorus, a 67 percent increase in sweetgum seedlings with large root collars was obtained by inoculation; however, inoculation had no effect on diameter growth when 200 pounds per acre of phosphorus was applied.

VESICULAR-ARBUSCULAR MYCORRHIZAE OF YELLOW-POPLAR: EFFECTS ON SEEDLING GROWTH IN FOREST NURSERY SOIL AND IN AN ARTIFICIAL MEDIUM. D. A. Starkey and M. F. Brown. Department of Plant Pathology, University of Missouri, Columbia, Missouri, 65201.

Studies were conducted to determine the feasibility of inoculating nursery soil and an artificial medium with vesicular-arbuscular (VA) mycorrhizal fungi to increase the growth of yellow-poplar (Liriodendron tulipifera) seedlings. Effects of three species of fungi were compared to define interspecific variations in growth response. Glomus fasciculatus, G. mosseae and G. caledonius were used to inoculate nureery plots at the State Forest Nursery in Licking, Missouri, and sterilized and non-sterilized nursery soil in greenhouse experiments. A positive correlation was noted between mycorrhizal infection levels and several growth parameters with seedlings grown in nursery beds. Responses to the test organisms were greatest in sterilized soil under greenhouse conditions with dry weight values up to 9.9, 4.5 and 3.5 times those of controls for G. fasciculatus, G. mosseae and G. caledonius, respectively. In non-sterile soil, dry weight values of 2.6 and 1.7 times those of controls were obtained for G. fasiculatus and G. mosseae respectively, while results obtained with G. caledonius were inconclusive. Containerized seedlings grown in a peat moss-vermiculite medium were employed to investigate the influence of available nutrients on G. fasciculatus and seedling development. On a dry weight basis growth of inoculated seedlings was twice that of controls under two nutrient levels. The greatest dry weight and infection levels were obtained with 236, 45, 120 ppm NPK, respectively. Data suggest that seedling growth can be significantly enhanced in bare root or containerized seedling production by inoculation with appropriate VA symbiotes.

DIFFERENTIAL RESPONSE AND FIELD PERFORMANCE OF CONTAINERIZED SEEDLINGS OF DOUGLAS-FIR, WESTERN HEMLOCK, AND PONDEROSA PINE INOCULATED WITH DIFFERENT ECTOMYCORRHIZAL FUNGI.

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Early results indicate that seedlings grown 10 months in inoculated peat-vermiculite respond in strikingly different ways to different fungi. For example, as compared to noninoculated controls *Laccaria laccata* depressed biomass production but increased the top/root ratio (T/R) of ponderosa pine; slightly increased both biomass and T/R of Douglas-fir; and strikingly increased biomass but reduced T/R of hemlock. On the other hand, *Pisolithus tinctorius* decreased biomass but did not affect T/R of pine; decreased both biomass and T/R of Douglas-fir; and strongly increased biomass while strongly decreasing T/R of hemlock. Inoculated seedlings have been outplanted for comparison of the different fungi on seedling survival and growth.

MYCORRHIZA DEFICIENCY IN REFORESTATION SITES OF THE NORTHWEST

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The history of a site--how and when it was harvested or burned, what kind of forb or shrub cover develops--may combine with weather vagaries, soil characteristics, and timing of seeding or planting to produce either temporary or long-term mycorrhizal deficiency. Current studies aim toward defining how, where and when this happens. Such sites are poor prospects for reforestation by see, and special attention should be given to tailoring mycorrhizae of seedlings to be planted on them.

The impact of crude oil on two mycorrhizal complexes of Salix rotundifolia from the Coastal Arctic Tundra. R. K. Antibus, A. E. Linkins and O. K. Miller. Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA. 24061.

The morphology, anatomy, respiration, and overall oxidative metabolism of the mycorrhizae of Salix rotundifolia Trantv. has been examined from soils impregnated with fresh crude oil at Barrow, Alaska and from soils immediately proximal to a natural oil seep at Cape Simpson, Alaska. Application of 5 or 12 l m^{-2} Prudhoe crude oil to Salix at Barrow on 1 July caused a 40-60 % decrease in mycorrhizal root respiration within 24 hr. Decreases in respiration levels of up to 85 % and lowered respiratory quotients continued throughout the season. Loss of the basidomycete dominated mantle seems to account for much of this change. The second season revealed levels of respiration in the 5 l m^{-2} oil treated mycorrhizae higher than those of the controls. Respiration in the 12 l m^{-2} oil treated mycorrhizae were also high. Light microscopic evaluation of thin sections and scanning electron microscopic observation indicate that significant structural differences exist in the re-established mycorrhizae.

Root tip counts, and light and scanning electron microscopic observation indicate that Cenococcum graniforme Sow. Ferd. and Winge is the dominant mycorrhizal associate of S. rotundifolia in the oil impregnated soils of the Cape Simpson oil seep. The structure and morphology of these ectomycorrhizae is quite different from those basidomycete dominated ectomycorrhizae of S. rotundifolia at Barrow. Respiration rates were higher for these mycorrhizae and respiratory quotients were also different. Fresh oil perturbation at the structural and respiratory levels was far less than that noted for Barrow mycorrhizae. The impact of oil on respiration at low temperatures was less severe on Simpson than on Barrow mycorrhizae.

EFFECTS OF ENVIRONMENTAL GASES ON RESPIRATION IN PINE ROOTS INFECTED WITH ECTOMYCORRHIZAE

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Respiration rate determinations were conducted on root segments of greenhouse-grown loblolly pine seedlings infected with ectomycorrhizae and on noninfected segments following exposure to low concentrations of two environmental gases, O_3 and SO_2. The determinations were made by differential respirometry on the composite structures of the infected root tip segments and on noninfected tips. Exposure to the gases was performed by flowing each test gas through the respirometer system. Respiration rates were made at 30-min intervals for 3 hr following a 1-hr exposure to each level of gas. The noninfected root segments were found to be more restricted by the test gases than those with ectomycorrhizae. The results indicated that development of ectomycorrhiza-root associations may afford some level of protection for the feeder root system of pine trees when exposed to either of the two environmental gases common in many polluted areas.

* to be presented by

INFLUENCE OF ENVIRONMENTAL GASES ON OXYGEN UPTAKE BY ECTOMYCORRHIZAL ISOLATES FROM PINE SEEDLING ROOTS

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Oxygen uptake determinations were made on two isolates of soil ectomycorrhizae commonly found on field-grown loblolly pine seedling roots following exposure to low concentrations of two environmental gases, O_3 and SO_2. The determinations were made by differential respirometric analysis. Exposure was accomplished by flowing the individual gases through the respirometer flasks containing culture growth of the isolates. Oxygen uptake was monitored at 30-min intervals for 3 hr following a 1-hr exposure to each level of gas. Both test gases were found to cause severe reductions in the rate of O_2 uptake. The most significant reductions occurred with SO_2 indicating that this environmental gas would be more damaging to the ectomycorrhizal population in the rhizosphere of loblolly pine seedlings than O_3.

* to be presented by

EFFECTS OF SULFUR DIOXIDE AND OZONE ON GROWTH OF ECTOMYCORRHIZAL ISOLATES FROM FOREST TREE SEEDLING SOILS

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Three isolates of Pisolithus tinctorius, an ectomycorrhizal soil fungus, found on loblolly pine and white oak forest tree seedlings were exposed to SO_2 and O_3 at two concentrations under laboratory conditions in a series of 3-hr exposure periods to determine the effects of these environmental gases on colony formation and growth. All three isolates showed inhibition of growth of aerial hyphae when exposed to O_3, while a stimulation was found with certain levels of SO_2. A few minor differences in colony formation and growth were also observed between the three different isolates.

* to be presented by

EFFECTS OF SULFUR DIOXIDE AND OZONE ON SPORULATION AND GROWTH OF ECTOMYCORRHIZAL-ASSOCIATIVE AND SUSPECT ROOT SURFACE ISOLATES FROM PINE TREE SEEDLINGS

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Selected soil fungus isolates associated with ectomycorrhizal formations in the rhizosphere of 2-3 year-old field grown loblolly pine tree seedlings were exposed to SO_2 and O_3 at two concentrations under laboratory conditions in a series of 3-hour exposure periods to determine the effects on sporulation and colony growth. Ozone showed complete inhibition of sporulation on isolates of Trichoderma viridae and Penicillium notatum. SO_2 significantly stimulated sporulation of T. viridae at the low levels but inhibited sporulation at the high levels, while little effect was found with this gas on P. notatum. Four of five ectomycorrhizal-suspect isolates showed significant inhibition of colony formation with O_3 in the first exposure series followed by periods of recovery. Most of these isolates showed significant inhibitions in growth to at least one concentration of SO_2 in the first exposure series with only one isolate showing continued inhibition throughout the study.

* to be presented by

The Effect of Hydrogen Chloride Gas or Ozone on Mycorrhizal Citrus

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Troyer citrange citrus were grown from seed and transplanted to individual pots of autoclaved loamy sand one week after germination. During transplanting, half of the seedlings were inoculated with 1450 spores of Glomus fasciculatus, an endophytic mycorrhizae. The citrus, grown in a glasshouse equipped with activated charcoal air filters, were fertilized weekly with a nutrient solution deficient in phosphorus. At 5, 12, and 16 weeks after germination, sets of 10 mycorrhizal and 10 non-mycorrhizal seedlings were exposed to 100-140 mg/m^3 hydrogen chloride gas (HCl) for 20 minutes or to 2 mg/m^3 ozone for 4 hours. Unexposed sets served as controls. The young citrus seedlings were tolerant to the high pollutant concentrations. HCl produced some burning of leaf tips and edges, but no visible symptoms were noted with the ozone treatment. The seedlings were harvested one week after final pollutant treatments and plant height, leaf, stem, and root dry weights, and the number of mycorrhizal spores associated with each seedling were recorded (Table 1). The control and HCl-treated mycorrhizal citrus were taller and heavier than non-mycorrhizal seedlings. The ozone-treated mycorrhizal plants, however, had roots which were lighter than their non-mycorrhizal counterparts. No significant difference was found between mycorrhizal and non-mycorrhizal ozone-treated plants in the other weight and height measurements. No spores were found associated with non-inoculated citrus seedlings. Of the mycorrhizal plants, the ozone-treated plants had significantly lower spore numbers than did the control or HCl-exposed seedlings (50% and 58% reduction, respectively). This study indicates that ozone reduces mycorrhizal spore production. HCl gas does not seem to affect the mycorrhizae; on the contrary, the fungus may offer some protection to the plant.

Table 1. Summary of harvest data on mycorrhizal and non-mycorrhizal citrus seedlings exposed to HCl gas or ozone

	Pollutant					
	Control		Hydrogen chloride		Ozone	
Mycorrhizae	+	-	+	-	+	-
Height (cm)	31.2	22.6	23.6	15.1	19.6	18.2
Dry weight (gm)						
Leaf	0.81	0.74	0.74	0.52	0.54	0.54
Stem	1.42	1.22	1.02	0.70	0.89	0.75
Root	1.80	1.77	1.12	0.96	1.13	1.33
Total	4.03	3.73	2.88	2.18	2.56	2.62
Spores (no. per 200 cc soil)	1064.3	0	905.2	0	528.4	0

ENDOGONACEAE AND "ENDOGONACEAE-LIKE" SPORE ASSOCIATES OF YELLOW POPLAR IN SOME SOUTHEASTERN FOREST TREE NURSERIES AND SELECTED FIELD SOILS

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Using a modified "flotation-adhesion" technique yellow poplar seedbed soils from nine southeastern forest tree nurseries were evaluated with respect to types and relative abundance of Endogonaceae spores. Soils from two widely divergent "field" sites, a cornfield and a 36-year-old yellow poplar plantation, were also evaluated. Effectiveness of methodology was confirmed by a rather surprising consistency between repeated spore counts.

By far the most abundant "Endogonaceae-like" chlamydospore recovered was a spore of uncertain taxonomic affinity. Believed to be identical to or a variety of Mosse's "crenulate spore," Mikola's "E-strains" and/or Wilcox's "BDG-58" this spore was recovered from 58% of the nursery soil samples representing 6 of 9 nurseries and 5 of 7 states. In soil samples where it occurred this spore constituted an average of more than 80% of the total spores recovered. At the Ralph Edwards Nursery in Morganton, NC, it outnumbered known Endogonaceae spores by a margin of better than 9 to 1, averaging in absolute numbers nearly 300 spores/gram of soil (ovendry weight basis). This very distinctive spore was not recovered from either of the "field" soils. It is not known whether this spore is symbiotic with yellow poplar, although by virtue of its apparently common association with nursery grown seedlings the possibility merits investigation.

The most abundant and frequently recovered spore known to belong to the Endogonaceae was apparently a variety (or varieties) of Glomus macrocarpus. Occurring in all nine nurseries chlamydospores of this fungus were recovered either as individuals or in clusters of up to as many as eight. Diameters of individual spores ranged from 44 to 206 μ with average diameters ranging from ca. 70 μ in Parsons, W, Va. to ca. 145 μ in Augusta, Va.

Predominant spore types recovered from the yellow poplar plantation included Glomus macrocarpus (var. geosporus), G. Fasciculatus and a Glomus sp. apparently not previously described. Azygospores of Gigaspora sp. were identified infrequently in several, but not all, of the nursery soils as well as the soil from the yellow poplar plantation. Gigaspora gigantea was the predominant spore type recovered from the cornfield soil. For the most part, all soils sampled contained varying numbers of miscellaneous Endogonaceae spores (primarily Glomus sp.) not easily assigned to species. In addition, several of the sample soils contained occasional "spores with internal spores."

Individual recoveries of known Endogonaceae spores ranged from a low of 1 to a high of 107 spores/gram of soil. Recoveries from the cornfield and yellow poplar "field" sites averaged 54.2 and 59.5 spores/gram respectively. Endogonaceae spores recovered from 8 of the 9 nursery soils, on the other hand, averaged from 1.5 to 23.0 spores/gram. The ninth nursery soil (Morgan Co., Ky.) had received no soil fumigation treatment for three years prior to sampling and spores recovered at this location averaged 50.7 per gram of soil.

Analysis of variance and computation of appropriate LSD's confirmed that Endogonaceae spore populations in the two "field" soils and the Morgan Co., Ky. nursery soil were significantly higher ($P \leq 0.05$) than those in the eight nursery soils which had recently received standard applications of various soil fumigants. These data corroborate reports regarding detrimental effects of soil fumigation on endomycorrhizal fungi and indicate that southeastern forest tree nurseries may be characterized by low endomycorrhizal inoculum potentials.

OCCURRENCE OF VESICULAR-ARBUSCULAR MYCORRHIZAE ON SHRUBS AND GRASSES IN STRIP-MINE REGIONS OF WYOMING

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Various grasses (rhizomatous Agropyron species, Elymus, Panicum, Andropogon, Oryzopsis), forbs (Salsola kali, Halogeton, Melilotus) and shrubs (Atriplex and Artemisia species, Ceratoides, Lupinus, Sarcobatus, Cercocarpus) from native and mining-disturbed soils in the Hanna and Shirley basins of central Wyoming were examined for vesicular-arbuscular mycorrhizae using quantitative methods.

Species from native sites generally were highly mycorrhizal: % infection in the perennial grasses ranged from 44 to 96% of the 100 1 mm root segments examined per site and roots of all shrub species surveyed supported Endogonaceae infection. The primary symbionts are species of Glomus and Gigaspora.

In mining-disturbed areas: 1) weedy annuals which typically invade immediately after mining and seeding were non-mycorrhizal; 2) naturally recurring perennials on orphaned spoils (non-revegetated spoils abandoned 20 or more years ago) were mycorrhizal; 3) on a north-facing, 1975-seeded raw spoil slope -- with denser Salsola cover and presumably better growth conditions than on the south-facing slope -- mycorrhiza infection in Agropyron species was less than half that on the south-facing slope; 4) seeded Atpiplex on the 1975 raw spoil was non-mycorrhizal; and 5) % infections of native and introduced species on seeded and stock-piled topsoils generally were higher than on seeded raw spoils, but were below % infections in undisturbed sites.

Our preliminary survey suggests that if non-mycorrhizal invaders persist, mycorrhiza inoculum (perhaps essential to competing native species) will decline because of insufficient propagation on host plant roots.

ECTOMYCORRHIZAE IN POLYGONUM VIVIPARUM L.

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The presence of ectomycorrhizae in Polygonum viviparum L. was signalled by Hesselmann in 1900 and confirmed by Peyronel (1930, 1937). Polygonum viviparum, an artic-alpine herbaceous species, is frequent and widespread in meadows and pasture-lands of Prealps and Alps at 1500 up to 3000 m; it is one of the few green herbaceous plants having ectomycorrhizae.

In the course of an investigation on the ecology of this species in Piedmont (Italy) alpine valleys, I was able to examine the mycorrhizae of numerous specimens collected at all vegetative stages and at different altitudes.

In the observed specimens, sixteen different types of ectomycorrhizae are found, recognizable for the morphological features of the symbiotic fungus. The mycorrhizal types are variously associated together: two, three, until five different types are found in a single specimen.

The most widespread symbiotic association is Polygonum viviparum + Cenococcum graniforme, present in 24 specimens, whose features are known.

Moreover, another interesting ectomycorrhizal type is observed in 9 specimens. These mycorrhizae show a strong partnership with fruit bodies of Russula emetica Fr. var. alpestris Boud.; they are simple, smooth, white-greyish, 0.5 - 1.5 mm long and 0.25 - 0.35 mm wide. The mycoclena is typically pseudoparenchimatous, hyaline, 20 μ thick, with polygonal outline hyphae in surface. The Hartig net envelops a single layer of lenthened cells, but penetrates into the same cells. This particular behaviour of the Hartig net was already described in connection with other few ectomycorrhizal Basidiomycetes (Ectomycorrhizae, Marks and Kozlowski 1973). The regular presence of Russula emetica var. alpestris carpophores, the close likeness between the mycelium - hyaline, septate, 2 - 2, 6 μ in diameter - forming these mycorrhizae and that one at the bottom of the carpophores, the finding, even if sporadic, of formations similar to cystidia both on the mycoclena and at the basis of the carpophores, can tentatively identify the symbiosis Polygonum viviparum + Russula emetica var. alpestris.

Abstract

(Third North American Conference on Mycorrhizae)

PERSISTENCE AND FRUITING OF ECTOMYCORRHIZAL FUNGI IN PUERTO RICO

by

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In 1959 we verified the successful use of imported soil inoculum for establishing ectomycorrhizae on *Pinus elliottii* in Puerto Rico. During subsequent visits in 1966, 1967, and 1976, we studied the persistence and fruiting of species of ectomycorrhizal fungi in established pine stands. Sporophores of the following Basidiomycetes were collected in association with *Pinus caribaea* trees that ranged from 6 to 16 years old: *Pisolithus tinctorius, Rhizopogon* sp., *Russula brevipes, Scleroderma aurantium, S. geaster,* and *Thelephora terrestris.* Several morphologically distinct types of ectomycorrhizae were collected and cataloged. Additional studies on the persistence and possible succession of mycorrhizal fungi on species of *Pinus* in Puerto Rico would be very profitable but should be made on a more frequent and regular basis.

The mycorrhizae of pioneer species found on bituminous stripmine spoil in western Pennsylvania

Analysis of 43 species of volunteer plants on a 3 year old bituminous stripmine spoil revealed that 81% were mycorrhizal. Of the 35 mycorrhizal species, 28 were found to have endomycorrhizae, 5 ectomycorrhizae, and 2 species had ectendomycorrhizae. The endomycorrhizal species were represented by only 7 families with the Compositae and Gramineae having the most species. Those that were ectomycorrhizal were all tree species. Two annual grasses had ectendomycorrhizae. All 8 species that were nonmycorrhizal were herbaceous and 6 were annuals. Three of these annuals were classified in the Polygonaceae.

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ABSTRACT

Some Observations on the Ecology of Arctic Mycorrhizal Fungi
by
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Basidiomycetes and Ascomycetes in Alaskan tundra are distributed amoung 17 families including 30 genera with fewer than 110 species. The semiaquatic habitats such as low center polygon troughs, polygon basins and inundated wet meadows have few mycorrhizal plant species and most of those are associated with endomycorrhizal fungi. Available evidence indicates that Basidiomycetes are greatly reduced in certain high moisture soils. On dryer study sites in coastal Arctic tundra only 7 fungus families and 11 genera represented by 22 species appear to be ectomycorrhizal associates of Arctic plants. The increasing diversity of mycorrhizal fungi is traced as one moves south from the Arctic Coast and the presence and abundance of many Arctic plants increases. Endomycorrhizae in Alaskan tundra are largely vesicular-arbuscular with few septa. They are associated with both Monocotyledons and Dicotyledons and have wide host ranges compared to Arctic ectomycorrhizal fungi.

Carbon, nitrogen and phosphorus contents, along with growth and respiration rates, of both fruiting bodies and mycelium of mycorrhizal fungi differ somewhat from non-mycorrhizal fungi. The wider distribution of mycorrhizal fungi over the Arctic Archepeligo is reported and related to the intensive studies on several Arctic sites.

STUDIES ON MYCORRHIZA: I. Survey of natural forest tree species of North-Eastern India

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Abstract

This paper presents the preliminary observations on mycorrhizal association of fifteen tree species, viz: Mesua ferrea Linn., Cinnamomum cecicodiphne, Stereospermum chelonoides DC., Shorea robusta Goertn., Castonopsis sp., Gmelina arborea Linn., Schima wallichii Chois., Terminalia mericarpa, Dillenia pentagyna Roxb., Syzygium cumuni, Ficus glomerata Roxb., Artocarpus chaplasha Roxb., Diospyros sp., Amara wallichii and Meliusa velutina. These species make a natural subtropical moist deciduous forest spread over an elevation of nine hundred meters in the state of Meghalaya (India). All species were found to be infested with mycorrhizal fungi. The symbiotic roots were examined microscopically for endophytous and ectophytous mycorrhizal associations. Mostly the tree species, viz; Mesua ferrea Linn., Meliusa velutina, Diospyros sp., Amara wallichii, Schima wallichii Chois., Ficus glomerata Roxb., Castanopsis sp., Dillenia pentagyna Roxb., and Artocarpus chaplasha Roxb., were found to be endomycorrhizal having vesicular arbuscular mycorrhiza. Other species i.e., Cinnamomum cecicodiphne, Shorea robusta Goertn., Terminalia mericarpa, Steriospermum chelonoides DC., and Cmelina arborea Linn. exhibited ectomycorrhizal association. A comparative study was carried out for the morphology of mantle, hartig net, vesicular-arbusculae, color reaction and flurescence in long wave length ultra violet light.

Mycorrhizal fungi associated with *Festuca* in the western United States and Canada

by

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Vesicular-arbuscular mycorrhizal infection and associated mycorrhizal fungi were examined for *Festuca viridula*, *F. idahoensis*, *F. scabrella*, *F. thurberi*, *F. ovina* and *F. arizonica* occurring in *Festuca* dominated grasslands in the western U.S. and Canada. All plants were mycorrhizal. Nearly all had mycorrhizal infection in 75% or more of their fine root length. Although levels of infection were consistently high, spore numbers were generally low. No differences in the degree of infection between *Festuca* species or habitats were observed.

Eleven mycorrhizal fungi were identified and ranked by decreasing frequency as follows: *Glomus fasiculatus*, *G. tenuis*, *Gigaspora calospora*, *Acaulospora taevis*, *Glomus macrocarpus* var. *macrocarpus*, *G. microcarpus*, *Acaulospora scrobiculata*, *Glomus mosseae*, *G. macrocarpus* var. *geosporus*, *Sclerocystis rubiformis*, and an unidentified *Acaulospora* species. Multiple infections by two or more fungus species for individual plants were most commonly observed. The mean number of fungal associates per community site ranged from 2.7 species for *F. idahoensis* to 5.0 species for *F. arizonica*. No evidence for specificity of any of the mycorrhizal fungi for any particular *Festuca* host was observed.

DYNAMICS OF VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI IN MESIC PRAIRIE AND PASTURE SOILS

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Before any comprehensive studies can be done involving the function of vesicular-arbuscular (VA) endosymbionts in trophic relationships and mineral nutrient transfers of ecosystems, a more accurate picture must be obtained of the dynamics of endogonaceous fungal growth. This study was designed to examine seasonal patterns of root infection and spore development in adjacent grazed and ungrazed field sites in western Pennsylvania (Butler Co.). These include: (1) The Jennings "Blazing Star Prairie", Western Pa. Conservancy, which has been maintained by periodic burning or clipping for at least 25 years; (2) a heavily grazed cow pasture; (3) an ungrazed but annually harvested field.

Randomly selected soil cores (width 6 cm.; depth 15 cm.) were collected seasonally from each site. Roots were stained with trypan blue and a modification of the line-intersect method was used to assess the percent of root length with endomycorrhizal arbuscules, vesicles, or hyphae, and that which was uninfected. Spores and sporocarps of VA fungi were collected by wet-sieving and decanting followed by flotation in 50% glycerol. An effort was made to characterize (quantitative and qualitative) the spore population associated with each field site.

Evidence is presented to show that heavy grazing results in a significant reduction in per cent infected root length. This difference can be attributed primarily to the lower level of vesicular infection recorded in roots from the grazed site, since levels of hyphal and arbuscular infection were essentially the same in each site sampled. Relationships between VA infection levels, spore or sporocarp numbers and certain selected site parameters are discussed. The relative importance of site related vs. seasonal effects on VA propagule distribution will be considered.

Mycorrhizal Fungi Associated with Soils and Plants in Texas

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Thirteen mycorrhizal spore types were recovered from soil samples collected in North, South Central, and Southeast Texas. Spores were recovered by the wet-sieving technique from soils associated with plant roots of Arachis hypogaea, Citrullus vulgaris, Rubus sp., Carya illinoensis, Pinus sp., Baptisia sp., Allium sp., Sorghum vulgare, and Gossypium hirsutum. Mycorrhizal spores were recovered from all soil samples. Spores were also examined in situ in plant roots. Tentatively identified species include Glomus fasciculatus, Glomus spp., Gigaspora coralloidea, G. heterogama, G. calospora, G. gigantea, Sclerocystis rubiformis, S. coremiodes, and several unidentified species. One spore type, found in Baptisia soil, resembled the description given for a species recently isolated in Nigeria. Parasitism of spores was frequently observed, as were callosities. One sporangial spore type was observed that contained small spores individually attached to the inner wall of the sporangium. No true zygosporic forms were observed; however sporocarps, chlamydospores, vesicles, and azygospores were frequently encountered. Spores of several isolates were increased in pot cultures. Recovery of introduced spore types was erratic. In some cases a different spore type was recovered.

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Comments about ectomycorrhizae of spruce (Picea abies (L.) Karst) in southern Ardenne (Belgium).

Picea abies is an exotic tree widely planted in Belgium. The spruce ectomycorrhizae are of racemic type, they have a smooth or more or less granular sheet, silky, felty or pruinous. Sometimes, rhizomorphs or hyphal strands like the fungus mantel to other short roots or to the soil (this is frequent with the genus Cortinarius).

Colour of ectomycorrhizae is very variable, from white, white with yellow patches, yellow, lemon yellow, cinnamon, orange yellow, brown ocre, rusty brown, dark brown, black, olive yellow, pinkish white, pale lilac, blue grey, ...

Cortical cylinder of short roots is constituted by five cells' layers, with gennerally 1 or 2 tannin layers (rarely 3 or more). The central cylinder is diarch (2 xylem poles). It is generally plenty of starch grains. We rarely, noted starch in the endodermis or in the cortical cylinder.

The diameter of the short mycorrhizal roots varies between 300 µm and 700 µm. The fungus mantel's thickness varies between 20 and 80 µm. Upon cross-sections, you can often distinguish several different zones in the mantel: the internal zone is often less coloured and is constituted by hyphae more densely interwoven and which have a thinner diameter. We also observe mantels constituted by smooth hyphae and incrusted hyphae. We observe laticifers in the mantel of ectomycorrhizae formed by genus Lactarius. Oleiferous hyphae and "H" anastomoses can also be seen in different mycorrhizae's mantel.

Until today, several symbionts of Picea abies have been identified in our country, for example, Cenococcum graniforme (Sow.) Ferd. & Winge, Corticium bicolor Peck., Dermocybe cinnamomea (L. ex Fr.) Wunsche s.l., D. sanguinea (Wulf. ex Fr.) Wunsche, Tylopilus felleus (Bull. ex Fr.) Karst., Paxillus involutus (Batsch ex Fr.) Fr., etc.

Many ectomycorrhizae are still undeterminable on the field. We may say, that on surfaces of 1/2 hectare, we noted the presence of 17, 18, 22 and 23 presumed mycorrhizal species, that is an average value of at least 20 kinds of different mycorrhizae per 1/2 ha.

We generally, recognise old ectomycorrhizae at their dark colour, their grooved surfaces and their cells of the cortical cylinder that are no more turgescent.

Growths revivals that we can recognise at the light and swollen radicular tips, have been observed during the winter (November, December, January) as well as in spring (April).

Most of the ectomycorrhizae are localised in the superficial and humus layers of the soil.

MYCORRHIZAL ASSOCIATIONS: ECOSYSTEM MECHANISMS FOR LONG-TERM SUSTAINED PRODUCTIVITY

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Based on studies of aboveground and belowground dynamics of forest ecosystems within the warm temperate climate, three autotrophic-heterotrophic couplings appear successful, as evidenced by their persistence on the landscape. These are: (1) recalcitrant, deciduous ectotrophs; (2) succulent, deciduous endotrophs; and (3) recalcitrant, coniferous ectotrophs. Theoretically the fourth coupling would be a succulent coniferous endotroph; it does not occur. Several characteristics are associated with the three couplings. These lead to questions concerning their relative success within varying climatic zones, their clustering within favorable microclimates, and their response to and recovery from stress.

It is our hypothesis that these characteristics are not chance, but the result of evolutionary pressures on both the autotroph and mycobionts and upon the decomposer community at large. Further, we hypothesize that these three couplings have ecosystem level implications. Retranslocation of essential nutrients from senescing recalcitrant foliage and the existence of a persistent ectotrophic association implies a relatively closed, internalized nutrient coupling between the primary producer and mycobiont. This ectotrophic coupling operates temporally to minimize nutrient loss via drought stress. This coupling minimizes the importance of decomposer remineralization of detrital pools and subsequent mycorrhizal transfer to the producer. This coupling of decomposers to mycorrhizal uptake is typical of endotrophic associations.

ROLE OF MAN: PLANNED DISPERSAL OF TRUFFLE FUNGI BY MOVEMENT OF INOCULATED HOSTS

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Studies carried out in laboratory and field during the last fifteen years in Italy and in France have resulted in a rational truffle cultivation method. This requires action on the associated fungus, the plant host and the biotope. The basic principle is an increase in the potential soil inoculum.

Critical study of various inoculation techniques has shown that truffle mycorrhizae (Tuber melanosporum Vitt.), which inoculated mycorrhizal tree seedlings carry exclusively and abundantly, constitute the best inoculum. Production of mycorrhizal plants began in France in 1970 and became operational in 1973, with the organization by I.N.R.A. of a specialized production system.

Plants are inoculated in controlled-environment conditions. Seedlings, inoculated in the spring with suspensions of spores ready to germinate, pure fungal cultures, or excised roots carrying mycorrhizae, are raised in plastic pots in disinfected soil suitable for truffle cultivation. After one or two years growth, host plants are checked by sampling for their mycorrhizal status. If it is satisfactory, they are transplanted to favorable sites.

Among the many potential truffle hosts, Quercus pubescens Willd., Q. pedunculata Ehrh., Q. sessiliflora Salisb., Q. ilex L. and to a lesser extent Corylus avellana L. are used, because of their known "truffigenic" qualities. Seed is harvested from productive trees. The most vigorous seedlings, with spreading root systems and good adaptability to the soil and climate of future transplantation sites, are retained.

Selection of truffle strains is inseparable from selection of the plant host. At present the focus is on capacity for mycelium growing in vitro, competitive ability in the symbiotic phase, and geographic origin of fruiting bodies.

Various methods (better spore activation, growing plants in various hydroponic conditions) have recently further improved plant quality, especially in intensity and purity of mycorrhizal development and plant vigor.

It is interesting to note that in the wild, when the degree of mycorrhizal infection by the truffle is sufficient and the conditions are favorable, mycorrhizal development continues and even extends to surrounding plants. This permits use of young mycorrhizal seedlings for interplanting to inoculate the soil (Indonesian method). Two years after plantation in truffle sites, some trees already show the characteristic "burnt" areas, which normally precede fructification and usually appear around traditionally inoculated plants only after about ten years. This is an encouraging sign of the success of this inoculation method.

THE COMBINED EFFECT OF ORGANIC MATTER AND SEASON ON DISTRIBUTION OF ECTOMYCORRHIZAE IN NORTHERN ROCKY MOUNTAIN FOREST SOILS.

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On an annual basis, 95 percent of active ectomycorrhizae in Douglas-fir/larch forest soils are associated with soil organic matter, only 5 percent occur in mineral soil. Humus, decayed wood, and charcoal are the most important sites. Humus provides the major substrate throughout the year except during July and August. During this drier period, decayed wood supports the most ectomycorrhizae. The total quantity of soil organic matter, including humus, decayed wood, charcoal, and litter, has a direct relationship with the number of mycorrhizae throughout the growing season. Organic matter levels between 30 and 45 percent, of the upper 30 cm (12 in), are optimum. Above or below these levels the number of ectomycorrhizae are reduced. These results confirm the critical relationship between soil organic matter and site quality. In particular, they emphasize a critical contribution made by decayed wood during times of moisture stress.

TROPICAL TREE GROWTH IS MYCORRHIZA-LIMITED IN A SEDGE-FILLED PASTURE

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Overgrazed pastures in northeastern Costa Rica, a region of lowland tropical rainforest, are dominated by sedges. Natural forest regeneration after pasture abandonment is slow. Sedge dominance may diminish mycorrhizal inoculum availability, contributing to reduced tree seedling growth, because sedges do not associate with obligately-root-inhabiting vesicular-arbuscular mycorrhizal fungi.

I grew seedlings of Pithecellobium longifolium (H&B)Standl. and Stryphnodendron excelsum Harms with and without mycorrhizal fungi for 6 1/2 months before transplanting to adjacent 100 m^2 pasture sites. One site contained native pasture grasses and tree roots which extended from forest bordering two sides. The other site was farther from the forest, and was sedge-filled. The sites were similar in fertility. At comparable fertility levels, P.longifolium and S. excelsum seedlings grow when mycorrhizal, but almost stop growing when uninfected.

P.longifolium and S. excelsum seedlings previously inoculated with mycorrhizal fungi continued to grow after transplanting to both pasture sites. Uninoculated P. longifolium in the forest-edge site became infected and grew as well as inoculated seedlings in the sedge-filled site. Growth of uninoculated P. longifolium was slowest and most variable in the sedge-filled site. Uninoculated S. excelsum did not survive in either site. Growth improvement by mycorrhizal inoculation suggests that diminished indigenous inoculum in the sedge-filled site limited seedling growth.

THE ROLE OF MYCORRHIZAE IN INFLUENCING SUCCESSION ON ABANDONED EVERGLADES FARMLAND

By

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Mycorrhizae can influence the direction and floristic composition of succession on abandoned farmlands. Plants and soils were examined for mycorrhizae in five successional and two mature ecosystems in a large (c. 3000 ha.) area of abandoned farmland within Everglades National Park known as the Hole-in-the-Donut. The five successional stages were: 1) farmland disked 3 months prior to sampling, 2) 2.5-year-old-regrowth (dominated by Panicum purpurascens), 3) 2.5-year-old-regrowth (dominated by Ludwigia peruviana) burned 6 months prior to sampling, 4) 17-year-old-woodland (dominated by Myrica cerifera), and 30-year-old-forest (dominated by the exotic tree Schinus terebinthifolius). The two mature sites were: 6) a seasonally flooded glade and 7) a mature hardwood hammock forest. All sites except 4 and 7 are flooded from June through November of most years.

Of twenty plant species examined in each site, three in site 1 were found to have mycorrhizae, seven in site 2, four in site 3, twelve in site 4, twelve in site 5, none in site 6, and fourteen in site 7.

Dominance at each site was determined by biomass harvests in sites 1, 2, 3, and 6 and by basal area measurements in sites 4, 5, and 7. Mycorrhizal plants dominated all sites except the 3-month-old-field and the mature glade. The lowest percentage of mycorrhizal plants in the successional sites was found in the farmland disked 3 months prior to sampling. Between 3 and 30 months after abandonment, mycorrhizal plants dominate the community. The mycorrhizal status of the vegetation was not well correlated with soil pH, Ca, Mg, Mn, or PO_4.

Mycorrhizal fungi from each site were identified by planting soybeans in soil from each site to permit endomycorrhizal propagules in the soil to infect the soybean roots. The fungi were given sufficient time to fruit and the spores were then extracted from the soil and identified. Thirteen species of vesicular-arbuscular fungi were encountered by this method. Only one species, Glomus macrocarpus var. geosporus, was found in all sites while 9 species were found in only one or two sites.

No mycorrhizae were found in the plants of the seasonally flooded glade, indicating that this ecosystem may be non-mycorrhizal: a rarity in natural terrestrial ecosystems. Because mycorrhizal plants have an advantage in nutrient absorption over non-mycorrhizal plants, farming practices such as rock-plowing, disking, and the addition of Perlite and Vermiculite may have allowed mycorrhizal plants to establish in an area formerly dominated by non-mycorrhizal plants. The structure of the vegetation has changed from wet prairie to new ecosystems where hardwoods, which include exotic species, are conspicuous and dominant.

MYCORRHIZAL ASSESSMENT OF 7-YEAR-OLD SLASH PINE AND ASSOCIATED RHIZOSPHERE MICROBIAL POPULATIONS ON KAOLIN SPOIL

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Trees from existing slash pine plantings on kaolin spoil were separated into vigorously growing and poorly growing classes. Trenches 0.75 m X 0.30 m and 0.10 m deep, were dug beneath selected trees and amount of short roots per gram of lateral roots less than 2 mm in diameter were determined from this soil volume as well as percent total mycorrhizae and percent mycorrhizal types. Similar trenches were dug beneath trees on a slash pine plantation growing on an undisturbed site. No vigor classes were distinguished on this site.

Regardless of tree vigor, the predominant mycorrhizal type on the kaolin plantings was Pisolithus tinctorius, averaging 70% of the total mycorrhizae present. Total mycorrhizal percentage ranged from 85% to 95% on all kaolin spoil plantings, while the trees on the undisturbed site had an average of 59% total mycorrhizae. There were, approximately, twice as many short roots per gram of lateral root in the kaolin plantings over that of the undisturbed plantings. Although having lower total percent mycorrhizae, the undisturbed plantation had a greater diversity of mycorrhizal types (8) versus 3 to 4 for the kaolin spoil plantings.

Both rhizosphere bacterial and fungal populations tended to be lower in the poorly growing trees on the kaolin spoil plantings. Populations of rhizosphere bacteria and fungi of the undisturbed plantation, however, tended to be lowest overall. Similar trends were observed for rhizosphere nitrogenase activities of the kaolin spoil and undisturbed plantings as determined by the acetylene reduction technique. Populations of root-free soil fungi and bacteria tended to increase from poorly growing trees to vigorously growing trees on the kaolin spoil sites. Although, the highest populations of these organisms were found in the soil from the undisturbed plantation. No differences were found between soil nitrogenase activities of the kaolin spoil plantation vigor classes and the undisturbed plantations.

A SURVEY OF MYCORRHIZAL INFECTION IN A TROPICAL ENVIRONMENT

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A survey of tropical economic and forest species is being conducted to determine the frequency of mycorrhizal infection and its distribution in various soil types and successional stages. Initial results indicate that infection is common but not universal in amazonian forest species, with about 80% usually being infected. A species-specific survey is now being initiated on a one-hectare plot of lowland rainforest in which 179 tree species have been identified. Tropical economic species are usually infected but this survey is indicating the existence of possible mycorrhiza-related difficulties in silviculture and agriculture. Infection appears to be suppressed in certain environmental conditions, including conditions commonly imposed by cultural practices. A collection of spore types is being maintained and identification will be attempted as pot cultures mature. Several spore types are usually associated with each crop or vegetation type. At the time of this writing six distinct spore types are consistently being found; most of these appear to be Glomus species. Spore numbers are usually high in comparison to temperate zone soils, but a large range of values has been found in association with different soil types, burning treatments, and fertilization treatments. The overall range has been from 47 spores/ 100 g soil in river-deposited soils to 2200 spores/ 100 g in a fertilized cacau plantation.

DIRECT NUTRIENT CYCLING BY ENDOMYCORRHIZAE

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Evidence now exists which supports the early theory of direct nutrient cycling by endomycorrhizal fungi. Recent photomicrographs show the movement of ^{32}P from a dead leaf into mycorrhizal hyphae, and into living root cells. The verification of nutrient uptake from dead organic matter on poor soils by mycorrhizal fungi and transport of nutrients into living roots limits the types of plants which can be grown efficiently on depleted tropical soils and is important to the planning of the future development of the Amazon.

STUDIES ON THE VESICULAR-ARBUSCULAR MYCORRHIZAE OF EASTER LILY IN THE PACIFIC NORTHWEST

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The vesicular-arbuscular (VA) mycorrhizal fungi of commercially grown Easter lily were characterized. Monthly field soil and root samples were collected from March through September, 1975, from five fields in the coastal area of southern Oregon and northern California. Soil sievings were inoculated onto clover, onion and lily to increase mycorrhizal spore numbers and to facilitate identification. Four VA mycorrhizal species were found: Acaulospora trappei, A. elegans, Glomus fasciculatus, and G. monosporus. All four VA species infected Easter lily, clover, and onion. A. trappei and G. fasciculatus were the most frequently isolated species from all five fields.

Mycorrhizal infections in roots of field grown lilies were young and sparse in March and gradually increased until September when bulbs were harvested. Root systems became infected up to 75% with mycorrhizae in some fields. Highest infection levels were reached more rapidly in fields with four VA species present.

Yearling lily bulbs were inoculated in the greenhouse with soil and roots from onion, lily and clover trap plants. This inoculum contained spores of the four VA species previously mentioned. Two levels of mycorrhizal inoculum and three fertilizer rates were tested for their effects on lily growth. Controls were either non-inoculated or inoculated with a solution from the mycorrhizal inoculum which had passed through a 38 µm sieve to remove mycorrhizal spores. Plants given either the high or low level of inoculum did not grow as well as controls, apparently due to root rot caused by Fusarium oxysporum which had increased along with the mycorrhizae on trap plants, and was subsequently inoculated onto the lilies. More F. oxysporum was recovered from roots inoculated with mycorrhizae than from control plants inoculated with a sieving solution. None was recovered from the non-inoculated plants. The high fertilizer level reduced mycorrhizal infection and enhanced root disease. However, more mycorrhizal infection occurred in roots given high fertilizer than no fertilizer. The most mycorrhizae formed in plants given the low fertilizer rate. Controls were non-mycorrhizal.

In another experiment, Easter lily seedlings were inoculated with A. trappei in the form of spores and infected root fragments from an A. trappei-red clover pot culture. Controls were inoculated with a sieving solution made from the mycorrhizal inoculum. Plants inoculated with A. trappei grew significantly better based on fresh weight, and had a higher level of N, P, K, Ca, and Mg than non-mycorrhizal controls.

ENDOMYCORRHIZAL SYMBIONTS AND GREENHOUSE PROPAGATED YELLOW-POPLAR SEEDLINGS

ABSTRACT

Objectives were to determine specific endomycorrhizal smybiont infectivity in yellow-poplar seedlings, and to correlate infectivity with seed source. Sterilized growth medium was inoculated with spores and hyphae of Glomus mosseae, or G. fasciculatus, or G. mosseae + G. fasciculatus simultaneously with seeds from five sources.

After twelve weeks, five representative seedlings from each seed source were prepared for microscopic observation. Fifty microscopic fields ($3mm^2$) from each root were analyzed for the presence of intracellular hyphal coils, arbuscules, and vesicles. None were found in the uninoculated control. G. mosseae seedlings showed the most extensive endomycorrhizal infection; vesicles, arbuscules, and hyphal coils were observed in all inoculated treatments, however, infection was considerably greater with G. mosseae as the only smybiont. Infection was less when G. mosseae was mixed with G. fasciculatus. There were no effects attributable to seed source.

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ENDOMYCORRHIZAL SYNTHESIS BY GIGASPORA MARGARITA IN POINSETTIA[1]

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The roots of poinsettia (Euphorbia pulcherrima) cuttings became mycorrhizal when inoculated with azygospores of Gigaspora margarita either in the mist bed or at transplanting. At a fertility level of 250 ppm N-P-K applied once a week, there was significant stimulation of plant growth with heavy mycorrhizal synthesis characterized by profuse production of extra-matrical hyphae and external vesicle clusters; however, plants eventually became chlorotic. At a fertility level of 125 ppm N-P-K applied twice a week, plant growth was improved but mycorrhizal synthesis was repressed occurring only as scattered hyphae in the roots with no production of external fungal structures. Increasing the N-P-K application to 250 ppm applied twice weekly provided maximum plant growth, but prevented mycorrhizal development. The endomycorrhizal fungus stimulated rooting of cuttings by increasing root numbers and weight in the mist bed only when the cuttings were first treated with Rootone, with the effect being considerably greater than that induced by the hormone alone. Mycorrhizal cuttings were better able to withstand transplant shock under high temperatures than non-mycorrhizal cuttings. Thus, the main beneficial effects of endomycorrhizal synthesis occurred when plants were grown under marginal fertility and moisture stress conditions.

[1]Based on a portion of senior author's M. S. Thesis.

Comparative Studies of Vesicular-Arbuscular Mycorrhizal Fungi on Soybean. D. E. Carling, R. A. Brown, A. L. Rowell and M. F. Brown. Department of Plant Pathology, University of Missouri, Columbia, Missouri 65201.

Different species of vesicular-arbuscular mycorrhizal fungi (VAM) were evaluated for their ability to stimulate soybean growth. Several species of VAM were isolated from local soils and inoculum was increased in pot culture. These inocula were used in subsequent growth experiments conducted in the greenhouse. Similar growth experiments were conducted with species of VAM acquired from other parts of the U. S. Of the locally collected species, Glomus mosseae, Glomus fasciculatus and Glomus caledonius were the most effective at enhancing soybean growth, inducing total plant dry weight increases of 316%, 235% and 213% of controls, respectively. Gigaspora margarita was considerably less effective producing a 144% increase in total dry weight. Isolates of G. mosseae, G. fasciculatus and G. caledonius from other parts of the U. S. induced growth increases similar to those of local isolates, while a North Carolina isolate of Gigaspora gigantea increased plant dry weight equivalent to 320% of controls. Soybean variety Williams (maturity group III) was used in the above growth studies. However, representatives from maturity groups I (A-100), IV (Clark), V (Hill) and VII (Bragg), responded similarly to the same species of VAM. Electron microscopic examination revealed no fundamental ultrastructural differences between the VA mycorrhizae of soybean and other plants. Minor degrees of difference appear with respect to host response to arbuscular breakdown. No ultrastructural differences were detected between the mycorrhizal species on soybean examined to date. It appears that factors other than structural interactions are responsible for differing growth responses of soybeans.

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SPORE GERMINATION OF ENDOMYCORRHIZAL SPECIES

ECOTYPIC VARIATION

Germination has been found to vary considerably within the genus Glomus. Ecotypic variation has been proposed as an explanation for the variable germination observed within species. An ecotype of Glomus mosseae obtained in eastern Washington germinates 70-90% on water agar, whereas ecotypes from western and eastern Oregon rarely germinate over 10% on water agar. Scanning electron microscopy shows great morphological variability between ecotypes of G. mosseae.

GERMINATION IN NONSTERILE SOIL

A technique involving nonsterile soil has been adapted to facilitate observation of spores placed in soil. Germination is 80-100% and readily observed. Experiments have been designed to test by this technique the influence of hosts vs. nonhosts on germination and infection morphology as well as the influence of environmental factors such as temperature, pH, soil fertility, and rhizosphere and mycosphere organisms.

VA MYCORRHIZA-SPECIFIC ALKALINE PHOSPHATASE ACTIVITY LINKED TO GROWTH RESPONSES IN MYCORRHIZAL PLANTS

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The role of the mycorrhizal fungus in improving the phosphorus uptake of plants showing growth responses to VA mycorrhiza formation is now widely recognised. Recent evidence indicates that both the transport of phosphate ions within the fungal hyphae from the soil to the host root and their subsequent transfer into the host cell involve active processes. This diversity in the mechanisms of phosphorus uptake by a mycorrhizal plant is accompanied by modifications in the soluble enzymes concerned in the phosphorus metabolism of the roots.

Analyses, after separation by gel electrophoresis, of the soluble acid and alkaline phosphatases extracted from mycorrhizal and non-mycorrhizal roots has revealed the presence of additional phosphatases in the mycorrhizal roots. These are specific to the presence of the mycorrhizal fungus itself and their number and activity depend on both the fungal and plant species involved in the mycorrhizal association. The mycorrhiza-specific phosphatase produced in the mycorrhizal association *Glomus mosseae/Allium cepa* var. Topaze has been studied in detail. Its properties are those of an alkaline phosphomonoesterase (E.C. 3.1.3.1.) – activity optimum at alkaline pH ; inhibition by metal chelating agents, certain cations and orthophosphate ; requirement for Mg^{++} and Mn^{++} ions ; hydrolysis of phenyl and naphthyl phosphomonoesters ; inability to hydrolyse more complex phosphate esters.

Mycorrhiza-specific alkaline phosphatase activity is closely correlated with the mycorrhizal growth response, being a maximum when growth stimulation occurs and declining afterwards as plant development and infection continues. Furthermore, it has not been found in extracts of onion roots infected with an endomycorrhizal fungus which does not cause a significant growth stimulation. These observations suggest that the mycorrhiza-specific alkaline phosphatase may be closely linked to the assimilation of phosphorus in the mycorrhizal system.

ABSTRACT. Third North American Conference on Mycorrhizae.

Carbon Nutrition and Physiology of Orchid Mycorrhizas.

The orchid mycorrhizal system differs from ectotrophic (sheathing) and vesicular-arbuscular types in that the principal role of the fungus is to channel carbon source compounds from the soil into the host. This is easily demonstrated in seedlings. At present there is no firm evidence that the same process is important in adult plants.

Germination and early growth of orchids is a heterotrophic phase during which development is enhanced by the provision of sugars, amino acids, vitamins and growth factors. However, the growth of asymbiotic seedlings (protocorms) is extremely slow and starch accumulates extensively in their tissues. Protocorms infected by a suitable endophytic fungus will grow on a simple nutrient medium. They do not accumulate starch and their rate of growth is always much more rapid than that of asymbiotic controls in nutrient-rich media. The endophytic fungus may therefore be diverting nutrients into metabolic processes associated with growth rather than the formation of storage materials. When starch-rich protocorms are infected their starch becomes depleted, indicating that storage processes are reversed by the infecting fungus. Using material that was heavily pre-infected by an endophyte, ^{14}C-glucose was readily translocated into protocorms when supplied externally to the fungus. The label was found in the insoluble fraction within a few hours and the proportion of labelled insolubles increased over several days. Non-infected protocorms showed limited uptake of ^{14}C glucose and did not rapidly accumulate insoluble compounds. Transfer of metabolites from fungus to orchid may be principally necrotrophic digestion of hyphae, but biotrophic transfer cannot be entirely ruled out.

When green plantlets were fed with $^{14}CO_2$ there was greater incorporation of label into insoluble substances in infected material. Nearly all of the label remained in the stem, leaves and shoot apex, with very little (1 - 2%) passing to the rhizome even two or three weeks after feeding. The endophytic fungus therefore does not provide a sink for photosynthate. Mycelium of the fungus growing from infected rhizomes on to agar carried negligible amounts of label from protocorms previously fed with $^{14}CO_2$. There appears to be little or no biotrophic transfer of nutrients from orchid to fungus, which throws doubt on the hypothesis that the relationship is a true mutualistic symbiosis.

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ENHANCED CARBON TRANSFER BETWEEN ONIONS INFECTED WITH VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI.

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Carbon transfer between plants was studied using; (1) onions inoculated with Glomus etunicatus and (2) uninoculated onion controls grown in high phosphorus (P) and low P soils. The mycorrhizal and high P control onions were of comparable size. Each replicate consisted of two plants, a donor and a recipient. After 60 days the shoots of the donor plants were exposed to 50μCi $^{14}CO_2$ for 24 h. After 72 h. the roots and shoots were assayed for ^{14}C activity. Recipient mycorrhizal onions had significantly ($P<.01$) higher ^{14}C levels in the roots, but ^{14}C levels in the shoots were not different from the controls. Mycorrhizal and nonmycorrhizal shaded recipient plants had similar ^{14}C transfer. In a separate experiment, ^{14}C-glucose was applied to soil containing isolated G. etunicatus hyphae treated and nontreated with PCNB. Nontreated and PCNB treated hyphae translocated significantly ($P<.05$) more ^{14}C to roots than diffused to roots of non-mycorrhizal controls. Roots with PCNB treated hyphae contained significantly ($P<.05$) less ^{14}C than nontreated mycorrhizal roots. These results indicated that ^{14}C transfer between plants occurred and that active (cytoplasmic translocation) and passive (mass flow along hyphal walls) transport may be involved.

VA MYCORRHIZAL INFECTION IN THE CHENOPODIACEAE AND BRASSICACEAE

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Six species of the Chenopodiaceae and Brassicaceae were inoculated with Glomus fasciculatus and examined for vesicular-arbuscular (VA) infection. The test plants were grown with and without a known mycorrhizal host, either onion or citrus. After 10-12 weeks the roots were stained for VA infection. Only those plants grown together in the same pot with a known VA host became infected and only vesicles (chlamydospores) and hyphae were observed; no arbuscules were found. Chenopodium album and Raphanus sativus var. cherry belle had the highest infection (<5%). Less percent infection was found in: Chenopodium amaranticolor, C. quinosa, Beta vulgaris, Atriplex hortensis, Brassica oleracea var. copenhagen market, B. oleracea var. dwarf murden, B. juncea var. florida broad leaf, B. juncea var. green wave, and B. rapa. Hyphae were observed in the vascular cylinder of some infected roots, which is atypical of VA mycorrhizae. The infrequency of infection and the lack of arbuscules indicates that mycorrhizal formation in these species may only be possible when they are growing near a mycorrhizal host. It may be possible that hyphae from a VA host can only penetrate and infect weakened or dying roots of these species. The presence of vesicles should not necessarily be regarded as evidence that a particular plant is a VA mycorrhizal host.

ENDOMYCORRHIZAE AND SOIL FERTILITY INFLUENCE GROWTH OF SEEDLINGS FROM EIGHT SELECTED SWEETGUM FAMILIES

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Sweetgum seedlings from 8 mother trees were grown in fumigated soil with or without the endomycorrhizal fungus Glomus mosseae at four levels of soil fertility for one growing season in nursery microplots. The fertilizer levels were 140, 280, 560 and 1120 kg/ha of 10-10-10 fertilizer. All microplots received periodic top dressing of NH_4NO_3 totaling 1680 kg/ha during the growing season and the elemental Ca was standardized at 1120 kg/ha at planting. Nonmycorrhizal seedlings of all families had excessive mortality and those that survived did not exceed 5 cm in height regardless of soil fertility. Fertilizer treatments did not significantly influence growth parameters of endomycorrhizal seedlings. Infected upland ecotypes significantly outperformed bottom-land ecotypes in all parameters except height. These results demonstrate the obligate requirement of sweetgum seedlings in soil for endomycorrhizae. Implication of these findings in sweetgum tree selection programs and nursery production of seedlings is discussed. It was stressed that more attention should be directed towards selecting sweetgum from less fertile upland sites because progeny from these selections may gleam more benefits from the endomycorrhizal symbionts than progeny from selected trees obtained from fertile bottom-land sites. Adequate endomycorrhizal inoculum levels in nursery beds can result in the use of significantly less fertilizer in seedling production without a reduction in seedling quality.

ENDOMYCORRHIZAL INOCULATION DURING TRANSPLANTING IMPROVES GROWTH OF VEGETATIVELY PROPAGATED YELLOW POPLAR

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Ramets from 5 clonal lines of Liriodendron tulipifera in soil infested with an endomycorrhizal fungus, Glomus fasciculatus, showed height growth increases ranging from 240 to 892 percent over non-mycorrhizal ramets. Total dry weight of the endomycorrhizal ramets was increased from 47 to 163 percent over nonmycorrhizal ramets. All endomycorrhizal ramets attained plantable size in approximately 4 months after rooting, but almost all of the nonmycorrhizal ramets exhibited first season dormancy and thus failed to reach plantable size. The use of endomycorrhizae in overcoming physiological dormancy of vegetatively propagated hardwood cuttings is discussed.

INTERACTION OF MAGNESIUM, PHOSPHORUS AND MYCORRHIZAE IN RED CLOVER.
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Shoot and root dry weights and the extent of mycorrhizal colonization were determined for red clover plants grown in a sandy loam with Mg levels adjusted to 1.2, 10 or 30 percent of exchange capacity and P additions of 0, 25 or 75 µg/g above an initial Bray #1 P of 9 µg/g. $Ca(OH)_2$ was added to raise the pH of all treatments to 6.7. Half of the twenty replicates were inoculated with sievings of a Gigaspora gigantea stock culture. Fungal colonization increased when Mg deficiency was relieved but was not further affected by the superoptimal level of Mg. Colonization was inversely related to P fertility excepting the treatment extremely deficient in both elements, in which colonization was poorest. In this treatment mycorrhizae did not improve growth. The response to mycorrhizae formation was greatest at 25 µg/g P for all levels of Mg but was not significant at 75 µg/g. The effect of Mg on the yield response due to mycorrhizae varied with P level and followed no general trend. Within fertility treatments there were no significant positive correlations between extent of colonization and shoot dry weight. However, these were negatively related in the $P_{25}Mg_{10}$, $P_{25}Mg_{30}$ and $P_{75}Mg_{30}$ treatments. In the four treatments where the mycorrhizal response was not significant, the least colonization occurred. The data suggest that Mg fertility has a major effect on the mycorrhizal association and response only under limited conditions.

The Interaction Between Phosphorus Fertilization and Endomycorrhizae on Citrus

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The interaction between phosphorus fertilization and the mycorrhizal fungus Glomus fasciculatus was studied. An autoclaved, low fertility, loamy sand containing 7 ppm P was provided with all necessary soil nutrients except P. Superphosphate [$Ca(H_2PO_4)_2$] was incorporated into this soil at rates of 0, 6, 28, 56, 278, and 556 ppm of P. Seven replicate mycorrhizal and non-mycorrhizal Brazilian sour orange and Troyer citrange seedlings were grown at each P fertility level. Dry weights of mycorrhizal sour orange seedlings were significantly greater ($P = 0.05$) than non-mycorrhizal seedlings when fertilized with 0, 6, 28, and 56 ppm P (947, 1089, 347, and 253% greater, respectively). Mycorrhizal sour orange seedlings were not significantly larger than non-mycorrhizal seedlings fertilized with 278 and 556 ppm P. Dry weights of mycorrhizal Troyer citrange seedlings were significantly greater ($P = 0.05$) than non-mycorrhizal seedlings when fertilized with 0 and 6 ppm P (746 and 300% greater, respectively). Mycorrhizal Troyer citrange seedlings were not significantly larger than non-mycorrhizal seedlings fertilized with 28, 56, 278, and 556 ppm P.

The number of G. fasciculatus spores/100 cc of soil on plants inoculated with G. fasciculatus decreased with increasing P fertility levels. With sour orange, numbers of G. fasciculatus spores decreased from 1460 spores/100 cc soil on seedlings fertilized with 56 ppm P, to 617 spores/100 cc soil on seedlings fertilized with 278 ppm P, to 319 spores/100 cc soil on plants fertilized with 556 ppm P. With Troyer citrange, numbers of G. fasciculatus spores decreased abruptly from 1019 spores/100 cc soil on plants fertilized with 28 ppm P to 220 spores/100 cc soil on plants fertilized with 56 ppm P. No correlation could be made between % P in leaves, roots or stems of seedlings and the decrease in mycorrhizal spore production.

P% in leaves of both sour orange and Troyer citrange was enhanced by the mycorrhizal association at all P fertility levels. P% in non-mycorrhizal sour orange leaves never reached levels recommended for optimum growth, even in seedlings fertilized with 556 ppm P. P% in mycorrhizal sour orange leaves reached levels recommended for optimum growth when fertilized with 56 ppm P. P% in leaves of non-mycorrhizal Troyer citrange reached levels recommended for optimum growth in plants fertilized with 278 ppm P. P% in leaves of mycorrhizal Troyer citrange reached levels recommended for optimum growth in plants fertilized with 6 ppm P. It appears that mycorrhizal fungi may be substituted for applications of 278 ppm P (500 lbs P/acre) in the culture of sour orange; and mycorrhizal fungi may be substituted for applications of 56 ppm P (100 lbs P/acre) in the culture of Troyer citrange.

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My work has been with the effects of infection by *Glomus fasciculatas* of different strains of cotton. I've specifically looked at the uptake of phosphorus and zinc where these nutrients were limiting. I've found that the mycorrhizal association significantly increases the uptake of these nutrients when they exist below the critical levels in the soil but that there is great variation between strains of host plant in their response to such infection.

Given these results, I'm now taking a closer look at the rhizoshpere and particularly the release of zinc by clay minerals which bind it. I'm currently doing assays of rhizoshere extracts for organic acids which have been found important in these processes when metabolized by soil bacteria. And finally I'm extracting clay systems saturated with zinc with these rhizosphere extracts to determine if they displace zinc from they clay minerals more strongly than the extracts from control rhizosheres.

I will be finishing my doctoral thesis under Dr. H. E. Doner in Soil Biochemistry and Microbiology in June.

POLYPHOSPHATE TRANSLOCATION IN V-A MYCORRHIZAS

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Vesicular-arbuscular mycorrhizas can increase the supply of P to their hosts (Mosse, 1973; Tinker, 1975). This P is possibly translocated along the hyphae in the form of polyphosphate, which is sequestered in the vacuoles and hydrolysed prior to transfer to the host (Cox et al. 1975). In the light microscope, polyphosphate can be distinguished by its metachromatic reaction with toluidine blue at low pH and the appearance of polyphosphate in infected roots may therefore be followed (Cox et al. 1975).

Inoculated onions (Glomus mosseae/Allium cepa) were grown in 'Perlite' and fed Long Ashton growth medium adjusted to 10^{-6}M, 10^{-5}M or 10^{-4}M P and pH 6.5. Fresh root sections (30 - 40 µm) were cut on a bench-top freezing microtome and stained with 0.05% toluidine blue in 1% H_2SO_4. The sections were then rinsed and mounted in acid. The roots and sections were kept on ice throughout.

According to the level of applied phosphate, plants were grown showing various degrees of P deficiency symptoms and mycorrhizal infection. Metachromatic staining was always confined to the fungus and was thus never observed in uninfected roots. It was rarely recorded in the infected roots of P deficient plants, but was normally present in the infected roots of plants grown with a sufficient P supply. Some external hyphae always stained metachromatically irrespective of supplied P.

If a dose of P at higher concentration (complete Long Ashton - 1.3×10^{-3}M) was given to the P starved plants, metachromasy was observed within a few hours. Over the 24 h following the dosing, there was a dramatic increase in both the intensity of the metachromatic staining of the internal hyphae and in the number of the internal and external hyphae which were stained. Staining appeared to be confined to the vacuoles of the fungus and was, in general, first seen in the intercellular hyphae, appearing only later in the arbuscules and vesicles. Although young and mature arbuscules stained, senescent ones did not, nor did their associated intracellular hyphae.

The finding that polyphosphate is always detectable in external hyphae, but only found insdie theroot when the P supply is increased, supports the theory that there is a net translocation of polyphosphate from the external to the internal parts of the fungus, where hydrolysis of the polyphosphate may occur.

The synthesis of polyphosphate under different environmental conditions was investigated. Polyphosphate was found in plants in which the whole root system had been dosed, and the plant subsequently kept at 3°C. Since other work (Dr. K. Cooper, private communication) has suggested that extensive translocation of P does not occur at such low temperatures, it seems that polyphosphate can be formed at 3°C, P having been absorbed by the hyphae in or very near the root.

Cox, G., F. E. Sanders, P. B. Tinker & J. A. Wild. 1975. 'Endomycorrhizas'. Academic Press, London. p. 297-312.

Mosse, B. 1973. Ann. Rev. Phytopath. 11: 171-195.

Tinker, P. B. 1975. 'Endomycorrhizas'. Academic Press, London. p. 353-371.

AXENIC SYNTHESIS OF ECTOMYCORRHIZAE ON PINUS TAEDA BY BASIDIOSPORES OF CERTAIN MEMBERS OF THE AGARICALES

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Basidiospores were aseptically collected from basidiocarps of Amanita rubescens, Amanita citrina, Suillus hirtellus, and Suillus cothurnatus; the most frequent and abundant members of the Amanitaceae and Boletaceae found in association with planted Pinus taeda in the piedmont region of North Carolina (Orange and Durham Counties). The ectomycorrhizal nature of these fungi and the function of their basidiospores as effective inoculum was tested in two axenic culture systems utilizing P. taeda seedlings. The basidiospores were shown to be functional as propagules for synthesizing the ectomycorrhizal symbiosis in a vermiculite-peat-nutrient substrate test system (after Hacskaylo, Marx and Zak, Grand, etc.) whereas the basidiospores of only S. hirtellus and S. cothurnatus functioned in the root-hypocotyl explant test system (after Fortin). Ectomycorrhizae formed from basidiospore inoculum were morphologically (size, color, branching, etc.) and histologically (Hartig net, mantle, etc.) the same as those formed from mycelial inoculum of the respective fungi. Isolation from the spore-formed ectomycorrhizae yielded, upon culturing, fungi identical to stock isolates of the same species collected within the same locale. Development of ectomycorrhizal roots was observed as early as 1 month after inoculation with basidiospores of S. hirtellus in the explant test system. In tests conducted at a somewhat elevated level of soil and air temperature, basidiospore inoculum produced a higher frequency of ectomycorrhizae than did mycelial inoculum. The presence of a vigorous saprophytic contaminant (Aspergillus sp.) in several chambers did not affect ectomycorrhizal synthesis from basidiospore inoculum but it completely inhibited synthesis where mycelial inoculum was used. Basidiospore germination and subsequent mycelial development did not take place in the absence of the host seedlings. Non-inoculated seedlings produced both simple and bifurcate roots in the absence of a fungal symbiont but the short roots were non-mycorrhizal.

The studies described above demonstrate 1) that the selected Amanita and Suillus species are ectomycorrhizal with P. taeda, 2) that basidiospores of these fungi can function as effective inoculum for ectomycorrhizal synthesis, and 3) that certain axenic synthesis systems successfully used with mycelial inoculum are also satisfactory for use with basidiospore inoculum.

This type of information is prerequisite to a more detailed examination of host-stimulated germination of spores of ectomycorrhizal fungi and the dynamic establishment of the fungal-tree symbiosis.

Amino Acid Inhibition Analysis of Lodgepole Pine Ectomycorrhizae. R.C. France and C.P.P. Reid (Department of Forest and Wood Sciences, Colorado State University).

Ectomycorrhizae of field-grown lodgepole pine (Pinus contorta Engelm.) seedlings (fungal symbiont unknown) were analyzed for their specificity in amino acid transport between symbionts. Excised roots were investigated for NH_4^+-absorption and incorporation into simple ^{14}C-structures. Subsequent transport of the ^{14}C-amino acids was observed through use of the "inhibition technique" where competition between radioactive and non-radioactive forms of the metabolites was effected. Selected amino acids included aspartic acid, asparagine, glutamic acid and glutamine. Results show that ^{14}C-glutamic acid was the predominant amino acid form transported between symbionts with minor amounts of ^{14}C-glutamine detected. No ^{14}C-incorporation into aspartic acid and asparagine forms was observed.

Interspecies and Interstrain Growth Characteristics of Selected Mycorrhizal Fungi under Combined Temperature and Water Stress. R.C. France, M.L. Cline and C.P.P. Reid (Department of Forest and Wood Sciences, Colorado State University).

Pure culture studies of four mycorrhizal fungi were conducted under combined environmental stress regimes. Fungal species included Pisolithus tinctorius, Cenococcum graniforme, Thelephora terrestris and Suillus granulatus. Ecologically diverse strains of each species were subjected to various combinations of temperature (16, 21, 27, 32, 38°C) and water potential (0, -4, -8, -16, -32 bars) conditions. Water potential levels were adjusted with PEG 4000 with temperature control obtained in incubators. Growth was analyzed periodically by mycelial mat extension, and dry weight of fungal mat was determined at termination of the experimental period. Interstrain variation occurred in all four species in terms of overall growth rate and duration of lag phase of growth. Optimal growth at specific combined stress conditions was determined for each strain. Interspecific growth differences were greater than interstrain differences.

Ethylene in Relation to Infection of Douglas-Fir by Ectomycorrhizal Fungi and *Fusarium oxysporum*

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Abstract. Ethylene in soil has been suggested as a regulator of microbial activity resulting in fungistasis. Anaerobic microsites have been proposed as the source of ethylene, and soils with low oxygen and high organic matter have been identified as having higher ethylene levels. Furthermore, nitrate nitrogen suppresses ethylene production. Since ectomycorrhizae normally occur in undisturbed forest soils with high organic matter, low nitrates, and restricted aeration, it was hypothesized that the fungal symbionts of these mycorrhizae are favored by ethylene. Conversely, *Fusarium oxysporum* is known to disappear from the roots of seedlings planted in such sites. Therefore, ethylene gas, released from Ethrel which was periodically injected into the root zone of tube containerized Douglas-fir, was established at two levels (0.2-0.5 ppm and 0.7-1.5 ppm). Seedlings were inoculated with either *F. oxysporum* f. *pini* or one of five ectomycorrhizal fungi (*Cenococcum graniforme*, *Laccaria laccata*, *Pisolithus tinctorius*, *Heboloma crustuliniforme*, and *Rhizopogon vinicolor*), or both. The production of ethylene by non-Ethrel treated mycorrhizal- and pathogen-inoculated seedlings was also examined. Preliminary results indicated that *C. graniforme* mycorrhizae released more ethylene than non-mycorrhizal seedlings.

HIGH SOIL FERTILITY DECREASES SUCROSE CONTENT AND SUSCEPTIBILITY OF LOBLOLLY PINE ROOTS TO ECTOMYCORRHIZAL INFECTION BY PISOLITHUS TINCTORIUS

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Pinus taeda seedlings were grown in the Athens growth room for 10 weeks without ectomycorrhizae in soil under low and high rates of complete soil fertility, as well as these rates minus N, P, K, or Ca. Seedling growth, inorganic chemical content of needles, and soluble carbohydrate content of short roots were significantly affected by soil fertility, especially the high rates of N and P. Number and length of lateral and short roots were not affected by soil fertility. Sucrose and fructose content of short roots was lowest at high levels of N and P; glucose was not detected. Representative seedlings from the 10 fertility combinations were inoculated with vegetative inoculum of *Pisolithus tinctorius* and incubated for 19 to 21 days at a moderate level of complete soil fertility. Significantly more ectomycorrhizae were formed on seedlings in complete low fertility than on those from the high rates of N and P. Sucrose content of short roots determined prior to inoculation accounted for 85 percent of the variation in susceptibility of short roots to infection by *P. tinctorius*. These results indicate that high levels of N and P in soil decreases sucrose content of short roots of loblolly pine and decreases their susceptibility to ectomycorrhizal development by *P. tinctorius*. (Accepted Can. J. Botany).

EFFECT OF FERTILIZATION ON THE DEVELOPMENT OF ECTOMYCORRHIZAE AND GROWTH OF LOBLOLLY PINE SEEDLINGS GROWN IN SOIL INFESTED WITH PISOLITHUS TINCTORIUS AND THELEPHORA TERRESTRIS

BY

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Vegetative inocula of Pisolithus tinctorius and Thelephora terrestris were used to infest fumigated soil and form ectomycorrhizae with loblolly pine (Pinus taeda) seedlings in microplots under different fertilization regimes. After an eight-month growth period, fertilization with N or NPK stimulated formation of ectomycorrhizae by P. tinctorius by at least two-fold as compared to seedlings which were not fertilized. The abundance of ectomycorrhizae typical of those formed by T. terrestris was not affected by fertilization. Fertilization with N and NPK significantly increased the fresh weight of the seedlings by 24 and 20%, respectively, whereas artificial infestations of soil with the ectomycorrhizal fungi tested did not markedly affect growth of the seedlings. Chemical analysis of the foliage showed no effects of treatments on the concentrations of N and P. K content, however, was greater in seedlings grown in soil infested with P. tinctorius and fertilized with NPK than in other seedlings.

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ABSTRACT

STUDY OF INFLUENCE OF ECTOMYCORRHIZAE ON NITROGEN METABOLISM AND CATIONIC NUTRITION OF CONIFERS ON CALCAREOUS SOILS

Most of conifers are very sensitive to calcium carbonate in soil. Only, a few species are tolerant (Pinus halepensis, Pinus nigra nigricans, Cedrus atlantica).

With mycorrhizae, the growth of these tolerant species does not depend on calcium carbonate in soil, and calcium carbonate brings no modification of mineral nutrition, particularly of calcium and nitrogen nutrition.

Without mycorrhizae, the behaviour of these species is very different. The presence of calcium carbonate induces a generalized chlorosis and a very bad growth, with a high calcium accumulation in needles. Without mycorrhizae, the proteins synthesis is disturbed at two levels : when ammonium is transformed into amino-acids and when amino-acids are polymerized into proteins.

Now, we are studying the influence of mycorrhizae on the nitrogen metabolism of conifers (influence on nitrate reduction, on amino-acids synthesis and on proteins synthesis).

PECTINOLYTIC ABILITY OF SOME MYCORRHIZAL AND SAPROPHYTIC HYMENOMYCETES

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The mechanism of penetration of ectomycorrhizal fungi into the middle lamellae of the cortex of rootlets is still not completely known.

Whereas some authors have claimed that the fungi produce pectinolytic enzymes, others failed to prove the production of such enzymes.

Considering the possibility that pectinolytic enzymes might be induced in mycelia growing in the presence of pectin on an easily available carbon source (e.g. glucose) which is gradually depleted, the author used the following technique:

Different ectomycorrhizal species of the genus Boletus as well as some litter-decomposing species of the genus Marasmius were cultivated in a synthetic nutrient solution containing 0, 1% glucose $\pm$ 0, 5% pectin. Growth curves were determined, and the viscosity of the solutions was followed throughout the experiments.

In the mycorrhizal fungi the growth curves obtained with 0, 1% glucose and with 0, 1% glucose + 0, 5% pectin were almost identical. In both series, the fungi produced 6-8 mg of mycelium, corresponding to an economic coefficient with respect to glucose utilization of about 30-40%. The viscosity of the pectin-containing solution was not affected.

The growth rate and mycelial production of the litter-decomposing fungi were considerably enhanced in the pectin-containing solution. Here, the viscosity was reduced to the value found for the pectin-free solution.

Finally, galacturonic acid, the monomer of pectic acid, was found to be used as a carbon source by the litter-decomposing fungi, whereas the mycorrhizal fungi were unable to use this compound either alone or in the presence of a small amount of glucose.

The implications of the results with respect to ectomycorrhiza formation will be discussed.

Abstract

(Third North American Conference on Mycorrhizae)

TOTAL FATTY ACID PROFILES OF SEVERAL CLOSELY RELATED FUNGI

by

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Total fatty acid gas chromatographic profiles were determined for the closely related fungi *Corticium bicolor*, *Athelia bicolor*, *Athelia galzinii* (two isolates), *Athelia bombycina*, *Athelia borealis*, and *Athelia epiphyla*. The total fatty acid profiles for all organisms were different except for the two isolates of *Athelia galzinii*, which were the same.

INFLUENCES OF THE ECTOMYCORRHIZAE OF HEBELOMA CYLINDROSPORUM AND PISOLITHUS TINCTORIUS ON THE GROWTH OF YOUNG CLUSTER PINE

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Cluster pine (Pinus pinaster) is the main forest species in 'les Landes Francaises'. Roots of this tree show many ectomycorrhizae formed by various fungi.
The influences of the mycorrhizae of two of these fungi (Hebeloma cylindrosporum and Pisolithus tinctorius) on the growth and development of pine seedlings had been investigated in our laboratory. Experiments were performed on three different types of very acid soils from 'les Landes': 1) A_1 horizon of a humic podsol; 2) an eluviated muddy-sandy soil ('boulbene'); and 3) soils developed over coastal sand-hillock, poor in organic matter (3a - 0,55 percent) and very poor (3b - 0.08 percent). Three-month-old seedlings were transplanted in culture pots filled with autoclaved soil and then inoculated with pure cultures of fungi. In order to prevent excessive eluviation of soil and contaminations by air-borne mycorrhizal fungi, water was supplied to the pots by wicks in close contact with periodically watered carpet. Ten months after, the following observations were made:

Ectomycorrhizae of H. cylindrosporum were observed on the roots of almost all the seedlings inoculated with this fungi. Many basidiocarps of H. cylindrosporum were present at the base of the young pines, mostly on the soil very poor in organic matter (3b). Yet the degree of ectomycorrhizal development on roots was never very high with this species.

The ectomycorrhizal development by Pisolithus tinctorius was strong except for the seedlings growing on the second type of soil where less than one third of the young pines was mycorrhized. The weakest degree of ectomycorrhizal development was observed in the (3b) type of soil.

Fresh weights of aerial parts of the plants with ectomycorrhizae of H. cylindrosporum and/or P. tinctorius were 2 to 15 times greater than those of the control seedlings without mycorrhizae, and 2 to 4 times higher than those of pines mycorrhized by a complex inoculum collected in natural sites. In the soils (1) and (2), seedlings with ectomycorrhizae of H. cylindrosporum showed the most vigourous growth. In coastal sand-hillock, P. tinctorius was more effective.

These results corroborate the usefulness of ectomycorrhizae in reforestation of difficult sites. They indicate also that the efficiency of ectomycorrhizae is a function both of the ectomycorrhizal fungi and of the type of soil in which roots grow.

MYCORRHIZAL FUNGI AND SOIL FERTILITY AFFECTING GROWTH AND MINERAL NUTRITION OF WHITE PINE SEEDLINGS

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Tuber albidum Pico and the ecto-endomycorrhizal fungus are among the most widespread symbionts in the Piedmont conifer nurseries. The two fungi inoculated on white pine seedlings growing in pots, showed different ability in stimulating seedling's development according to the different types of soil. By means of chemical analysis one could ascertain that seedlings nonmycorrhized or mycorrhized with the different symbionts, have absorbed from the soil and stored in their tissues significantly different rates of the main mineral elements.

Tuber albidum produced, on the whole, the maximum development of seedlings; it gave the best results on forest soil rich in nutrients, and improved P and K assimilation. The ecto-endomycorrhizas on the contrary, stimulated the best development of pines growing on poor agricultural soil, and improved N and Na assimilation. Mg and Mn contents were generally higher in mycorrhized plants than in the controls, independently from the symbiont species; Mg and Ca contents were higher in seedlings grown on agricultural soil, which is richer in these elements. Factors under consideration did not affect Fe absorption.

CONTROL OF PLANT NUTRITION BY ECTOMYCORRHIZAL AND ENDOMYCORRHIZAL FUNGI

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The superior growth of mycorrhizal over nonmycorrhizal plants in low nutrient conditions has shown that the mycorrhizae function efficiently as nutrient absorbing organs. Segments of loblolly pine (Pinus taeda) roots infected with ectomycorrhizal fungi (Pisolithus tinctorius and Cenococcum graniforme) and uninfected roots were analyzed for elemental composition (magnesium, phosphorus, sulfur, chlorine, potassium and calcium). The chemical composition of cortex cells of vesicular-arbuscular (VA) mycorrhizae of Sorghum vulgare formed by Glomus mosseae and G. fasciculatus and nonmycorrhizal roots were also compared in elemental distribution. The values were obtained by a combination of scanning electron microscopy and electron microbeam technique.

Significant concentrations of the observed elements in both ectomycorrhizal and VA mycorrhizal fungi were higher than the nonmycorrhizal roots. This accumulation and transport of nutrients by ectomycorrhizal and VA mycorrhizal fungi to root cells is proposed as the major factor in the stimulation of plant growth under low fertility conditions.

The effects of mycorrhizal fungi and phosphorus levels on the root system development in germinating Pinus resinosa Ait.

By Richard Fohs Sohn, State University College of Environmental Science & Forestry, Syracuse, New York.

The initial development of the root system in Pinus resinosa Ait. was studied under controlled environmental conditions in a sterile test-tube-culture system utilizing ion exchange resin prepared to provide desired nutrient levels. Several mycorrhizal fungi were tested in the presence and absence of orthophosphate on the resin. Test tubes were simultaneously planted with seed and inoculated with fungus on an agar plug. Seven weeks after germination, few, if any, mycorrhizae had formed. However, developmental differences resulting from the various fungus and phosphorus treatments included the following: the number and density of long roots per seedling; the primary root length; the density of lateral root primordia on the primary root; and the development of second-order lateral root primordia. After further testing of various mycorrhizal fungi under controlled nutrient and moisture conditions, it should be possible to determine the extent to which initial root development influences later root system form and developmental characteristics. The cation and anion exchange resins provided adequate and precise nutrient supply while producing a well-buffered system of pH 5. The system could be sterilized by autoclaving without decomposing the resins and it could be easily analyzed for nutrient composition before and after experiments.

MYCORRHIZAE AND GROWTH OF WHITE FIR SEEDLINGS IN MINERAL SOILS WITH AND WITHOUT DUFF UNDER NATURAL CONDITIONS IN CALIFORNIA

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It has been observed that white fir [*Abies concolor* (Gord. et Glend.) Lindl.] seedlings survive and grow better in soil without, or with very light, duff. Our objective was to determine whether the better growth observed in seedlings occurring in mineral soil without duff (M) compared to those in mineral soil with duff (MD) could be related to the incidence of mycorrhizae in their root systems.

Naturally occurring M and MD seedlings were collected from five sites in the northcentral Sierra Nevada. Statistical analysis of the data showed that seedling dry weight was significantly related to total length of lateral roots and total number of mycorrhizal tips present on the roots. Both of these characteristics were significantly higher in the M seedlings than in the MD seedlings. There were significant differences in these variables among the five sites.

The data indicate that mycorrhizae play an important role in the growth of white fir seedlings, and that both growth and presence of mycorrhizae are favored by the absence of duff.

A Technique To Quantify Ectomycorrhizae On Forest Trees

Robert Anderson, Elwin Stewart, and Sagar Krupa[1/]

This ectomycorrhizae sampling technique has been used to evaluate over 5,000 trees during the past two years. Intensive field and laboratory checks has supported the conclusion that this technique provides an accurate assessment of the ectomycorrhizae situation in a given area with a substantial time savings.

NURSERY SAMPLING: 1) Randomly select two nursery seedling beds of the same age class. 2) Divide each bed into 40 equal units. 3) Randomly lift three or four trees from one location in each unit and pick the least damaged one for analysis. Attempt to get most of the roots, however, do not be overconcerned that the complete root system is not obtained since the analysis is in percent. 4) Wrap selected seedlings in a wet paper towel and place in labelled plastic bags, keeping the 40 seedlings from each bed separate.

FIELD SAMPLING: 1) Select 80 trees at random for examination. 2) If size permits, dig up the complete root system, wrap the roots in a wet paper towel and place in a labelled plastic bag. 3) With larger trees, carefully dig two meters of lateral roots from each of 80 trees.

TRANSPORTATION AND STORAGE: Refrigerate tree roots until examined or immediately ship by the fastest means to the place of analysis. The seedlings can be frozen if analysis will be delayed more than 30 days after collecting.

LABORATORY ANALYSIS: 1) Select 10 trees from each sample bed. 2) Examine feeder roots on the 20 seedlings or trees when total time required is less than 15 minutes, classify each feeder root as ectomycorrhizal (as indicated by a swollen appearance of the feeder roots--normally black, white, or golden) or nonmycorrhizal. 3) For trees requiring more than 15 minutes, use the following 10% method: (a) Cut all lateral roots from the main root; separate all lateral roots over 3" in length into individual sections. (b) Group the lateral roots into 3 categories by length. (c) Using strips of velcro taped to a bench, stretch the lateral roots end to end on the bench starting with the longest and ending with the shortest. (d) Measure the total length of the roots. Divide into 100 equal units and cut each 10th unit from the root system. (e) Place the roots in water and count the number of ectomycorrhizal and nonmycorrhizal feeder-roots in the 10% sample. (4) Select one ectomycorrhizal and one nonectomycorrhizal feeder root from each sample and examine for Hartig Net to insure that the mycorrhizae classification is accurate for the trees being examined. 5) A Standard Error of the mean less than $\pm$ 10% at the 95% level indicates sampling can be discontinued. Sample 20 additional trees if the error is greater. Repeat this procedure until 80 trees are examined.

This technique has been used on endomycorrhizae of other tree species with excellent results. The roots were cleared and stained for endomycorrhizae examination and quantification purposes.

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INTERACTIONS BETWEEN ENDOMYCORRHIZAE AND AZOTOBACTER ON PLANT GROWTH AND NUTRITION.

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Experimental work carried out in this laboratory has revealed that phosphate-solubilizing bacteria known to produce plant hormones could interact with vesicular-arbuscular endomycorrhizae in stimulating plant growth. The possibility of this cooperation being due to the activity of such growth regulators was suggested. This is now studied for *Azotobacter* able to produce substances of the type of auxins, gibberellins and cytokinins.

Lavender plants (*Lavandula spica* L.) were grown in two low-phosphate soils inoculated with resting spores of the endomycorrhizal fungus *Glomus mosseae*. The growth and N, P and K uptake and the degree of mycorrhizal infection was compared in plants also given different inoculation treatments with fractions of *Azotobacter* cultures.

The cell-free supernatant of the bacterial liquid culture, possessing the plant hormones, produced similar effects than that the whole *Azotobacter* culture (cell + supernatant) and that a mixture of commercial auxin, gibberellin and cytokinin at the same dose as they were produced by *Azotobacter*. Thus, can be deduced that growth regulators play a role in the above mentioned interactions.

Scanning electron microscopy of Red Pine mycorrhizae

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The purpose of this study was to gain more information on the microscopic and submicroscopic structure of ectomycorrhizae and ectendomycorrhizae. S.E.M. was employed because of its great range of magnification and because its great depth of field enables a three dimensional representation of the subject.

Red pine seedlings were grown in monexic culture with ecto- and ectendomycorrhizal fungi. The flask cultures were maintained for three or six months under greenhouse conditions. At the end of the growth period, the root systems were prepared for S.E.M. by gluteraldehyde fixation and critical point drying.

The specimens were viewed by S.E.M. for surface features including hyphal surface, fungal spores and mantle characteristics. Transverse sections of the long and short roots enabled observation of internal features of the mycorrhizas including the Hartig Net and intracellular hyphae. Significant differences in the appearance of these structures were noted between the two types of mycorrhizae. S.E.M. micrographs will be shown illustrating these features.

CHARACTERIZATION OF ASSOCIATIVE SOIL FUNGI WITH MYCORRHIZAE ON SPECIFIC FOREST TREE SEEDLINGS

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Preliminary to studying the effects of environmental gaseous contaminants on mycorrhizae on seedlings of loblolly pine and white oak forest trees, a study was made to characterize the associative soil fungi in the immediate rhizospheres of each tree. Isolation and characterization of these fungi were carried out on 2-3 year-old seedlings by using modifications of methods reported to study soil microflora-plant disease relationships. The results showed that certain genera and species of soil fungi were more predominant with each of the seedling types. Penicillium notatum and Trichoderma viridae were the predominant associative fungus isolates found. Penicillium species were found to be more common in the rhizosphere of white oak seedlings, while non-Penicillium species were more prevalent with the loblolly pine. Tentative identification showed thirteen possible mycorrhizal isolates associated with root tips of the two types of tree seedlings.

* to be presented by

Studies on Mycorrhizae of Ericaceous Plants

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Abstract: Ectendomycorrhizae on rooted cuttings of kinnikinnick (*Arctostaphylos uva-ursi*) were formed under nonsterile greenhouse conditions by the ectomycorrhizal fungi *Thelephora terrestris*, *Pisolithus tinctorius*, *Laccaria laccata* and *Rhizopogon vinicolor*. Zak had previously reported that these same fungi formed ectendomycorrhizae on kinnikinnick but under aseptic conditions. Initially the controls looked healthier and more vigorous than the mycorrhizal plants. When the experiment was terminated, however, those plants with mycorrhizae formed by *T. terrestris*, *L. laccata* and *P. tinctorius* looked healthier and had greater total growth compared to controls.

Several techniques were used to reisolate the fungi from kinnikinnick mycorrhizae formed by *P. tinctorius*, *T. terrestris* and an unknown basidiomycete: a direct plating method, a suspension-over-agar method, and a maceration technique. The results from all were negative. Because the two known fungi grow well in pure culture, and have been successfully isolated from ectomycorrhizae, we suggested that the ectendo relationship is more fragile and perhaps more obligate, making isolation very difficult.

When *R. vinicolor*, *P. tinctorius*, *L. laccata* and *T. terrestris* were mixed with *Phytophthora cinnamomi* inoculum in the soil, no protection of kinnikinnick against the pathogen occurred. This suggests that for pathogen protection to occur, mycorrhizae must either be formed first, or that pathogen protection cannot occur here, except by physiological means, since ectendomycorrhizae lack the thick mantle of the ectomycorrhizae where protection has been demonstrated.

INFLUENCE OF COMPOST ON ENDOMYCORRHIZAL DEVELOPMENT ON DIFFERENT PLANT SPECIES IN CONNECTICUT.

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Rhododendron, hemlock, sugar maple and corn were grown in containers of hemlock, sugar maple or corn compost. Mycorrhizal incidence in these plants was differentially affected by the differences in rooting medium.

EFFECT OF SORGHUM-SUDAN GRASS ON ROOTS OF NURSERY STOCK

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Several years ago some Wisconsin nurseries introduced green manure of sorghum-Sudan hybrids in the hope that the hydrocyanic acid from these plants would control Cylindrocladium floridanum and other root rot fungi. Casual observations suggested that this green manure depressed the development of root systems of nursery stock and decreased the abundance of mycorrhizal short roots. To examine this relationship, the following trials were conducted. Two crops of sorghum-Sudan were raised under greenhouse conditions. After 5 weeks of growth, the tissues were cut into approximately 2-cm segments and incorporated with the soil. Two weeks after incorporation of the second crop, in August, 1976, the soils were sown to red, white, and Monterey pines. All trees in sorghum-Sudan cultures suffered more than 50% mortality. The surviving 3-month-old seedlings revealed a drastic reduction of root systems and nearly total annihilation of mycorrhizal short roots. Some seedlings, particularly those of red and white pines, exhibited severe, burning-like injury of roots, similar to that inflicted by lead arsenate. The study indicates that plowing under of sorghum-Sudan and seeding or transplanting of nursery crops must be separated by a prolonged detoxification period with predominant temperature above 15^{o} C.

NB: Illustrated report.

Enhanced Rooting of Woody Plant Cuttings by Mycorrhizal Fungi

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Additional index words. *Arctostaphylos uva-ursi*, *Vaccinium ovatum*, Ericaceae, ectendomycorrhizae.

Abstract. When inoculum of ectomycorrhizal fungi was added to the rooting medium, the percentage of rooted cuttings and the root volumes on cuttings of kinnikinnick (*Arctostaphylos uva-ursi*) and huckleberry (*Vaccinium ovatum*) were significantly greater than those of the uninoculated controls. This enhanced rooting occurred before or in the absence of any mycorrhizal association. In some tests, inoculum of one fungus enhanced rooting of one cultivar of kinnikinnick, but not another, suggesting a specific interaction between the cultivar and the fungus. Of the thirteen fungi tested, only *Thelephora terrestris* formed ectendomycorrhizae in the propagation bed, although several others did so under other conditions. Possible mechanisms of this enhanced rooting phenomenon are discussed.

TREE HOST RANGE AND WORLD DISTRIBUTION OF THE ECTOMYCORRHIZAL FUNGUS PISOLITHUS TINCTORIUS

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The natural occurrence of Pisolithus tinctorius has been confirmed in 34 countries of the world and in 38 states in the United States. This ectomycorrhizal fungus is found associated with various tree species in nurseries, urban areas, orchards, forests and strip-mined spoils. Experiments have proven this fungal symbiont forms ectomycorrhizae with Abies procera, Betula pendula, Carya illinoensis, 11 species of Eucalyptus, 2 species of Picea, 30 species of Pinus, Pseudotsuga menziesii var. menziesii, 2 species of Quercus, and Tsuga heterophylla. Pisolithus has also been reported growing under natural conditions in association with 3 additional species of Betula, 2 species of Eucalyptus, 9 species of Pinus, 8 species of Quercus, Populus tremuloides, Pseudotsuga grandidenta, and Salix humilis.

This fungal symbiont has great potential in forestation efforts because of 1) the availability of techniques for artificially introducing it into nursery soils and container seedling substrates; 2) its ability to improve tree survival and growth in the nursery and the field; 3) its near world-wide distribution on a variety of sites; and 4) its broad host range encompassing many of the world's most important tree species.

Florida Citrus Mycorrhiza Research

S. Nemec[1]

Soils and or roots of citrus have been examined in 65 citrus groves and nurseries for VA mycorrhizae. So far Glomus fasciculatus, G. macrocarpus, G. mosseae, a small-spore form of G. caledonius, Sclerocystis sinuosa and Gigaspora margarita have been identified. Gigaspora margarita is the most common symbiont in groves. G. caledonius, G. macrocarpus and Sclerocystis sinuosa are more commonly found in groves than G. mosseae and a particular G. fasciculatus. Mean spore number in grove and nursery soils is about 12/gm soil with highest numbers present during the fall, however, most spores in soil samples are nonviable. Citrus nurserymen practice fumigation of seedbeds with methyl bromide, and to partially overcome stunting in seedlings, apply foliar or soil applications of phosphorus. In nurseries, lowest spore numbers were present in seedbeds on recently fumigated ground and highest numbers on seedbed planting 2 and 3 years old and on budded stock 2 years and over in age. Studies are in progress to determine the response of selected citrus rootstocks to VA mycorrhizae, the reproduction of the mycorrhizae on these rootstocks, and evaluate the tolerance of endomycorrhizae to soil chemicals.

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A CORRELATED HISTOLOGICAL AND ULTRASTRUCTURAL STUDY OF A VESICULAR - ARBUSCULAR MYCORRHIZA.

The complex pattern of relationship between the fungus and the host in the V.A. mycorrhiza of Glomus sp. with Ornithogalum umbellatum L. was studied with a correlated light and electron microscopic investigation.

The fungus from the root surface down to the deeper root layers shows these following stages:

- mycelium on the surface root
- hyphal coils in the outer layers of the cortical parenchyma cells
- extracellular hyphae, haustoria and arbuscules in the inner layer of the cortical parenchyma cells
- inter/or extracellular fungal vesicles, scattered in the different layers of the root cortex.

At the ultrastructural level the most remarkable features as concerns the fungus are walls with differential thickenings and an active protoplasm with frequent highly vacuolated areas, sometimes endowed with dense granules.

The host shows unusual plastids -like chromoplasts, hypertrophic nuclei and sometimes wall ingrowths in the exodermic cells.

At the interface of the complex fungus-host we can observe an amorphous matrix laid down in the space between the host plasmalemma and the wall of the intracellular fungus. Cytochemical data suggest that this matrix is formed by the host material and it is similar to a primary wall.

Hence the functional meaning of the different cytological areas can be discussed in ralation to the comples organization of the V.A. mycorrhiza.

A Simple Plate Method to Quantitate VAM Spores in Soils

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ABSTRACT

Spore extraction techniques have often been used to study soil mycorrhizal species and populations. The quantitative aspects of five soil extraction methods for vesicular-arbuscular mycorrhizal (VAM) spores were compared using a soil with a relatively high population of spores.

In a preliminary study, extraction methods consisting of an adhesion-floatation technique, a wet-sieve/sucrose centrifugation method, differential sedimentation of spores in gelatin columns and a plate method were evaluated. Spore counts per gram of moist soil between 150 and 300 µm were 74 for the plate method, 14 for the adhesion-floatation method, 20 for the wet sieve/sucrose centrifugation method and 14 for the gelatin method. VAM spore counts from the plate method were statistically higher than other methods at the 5% level.

Further comparisons were made between a wet-sieve extraction, a modified wet-sieve technique, and the plate method. Counts of spores per gram of moist soil between 100 to 300 µm were 70, 35, and 47 for the plate, wet-sieve, and modified wet-sieve methods, respectively. Again, spore counts from the plate method were significantly higher than the wet-sieve methods at the 5% level.

In a third experiment, spore counts as a function of spore size, were compared for: 50 to 100 µm, 100 to 150 µm, 150 to 300 µm, 100 to 300 µm, >300 µm and total counts per gram of moist soil. For all size comparisons, the plate method gave approximately twice the spore count as did the modified wet-sieve method and about 4 times that obtained using the wet-sieve method. These differences were significant at the 5% level.

Observations made in these studies indicate that the plate method may provide a more quantitative recovery of VAM spores from soils containing high density spore populations. In low spore population soils, the plate method may not be sufficiently sensitive unless a large number of samples are counted. Further investigations are being performed to determine the applicability of the plate method for routine use with variable VAM spore populations. Time and cost comparisons for the plate and wet-sieve methods are also being evaluated.

SMALL MAMMALS AS VECTORS OF HYPOGEOUS MYCORRHIZAL FUNGUS SPORES

James M. Trappe, Pacific Northwest Forest & Range Experiment Station
Forestry Sciences Laboratory, 3200 Jefferson Way,
Corvallis, Oregon 97331

Hypogeous fungi (truffles and false-truffles) are major components of the ectomycorrhizal flora of the western U.S. Because they fruit in the soil and are thereby protected, they are more dependable sources of natural inoculum than the mushroom-forming fungi, which often abort if the fruiting-season weather turns hot and dry or frosty. The hypogeous fungi are dispersed by mice, squirrels, and other small mammals, which dig them out and eat them. Many such mammals wander between clearcuts or burns and adjacent forest, eating hypogeous fungi in the forest and defecating the viable spores in the openings as little packets of inoculum.

Effects of ecto-, ectendo-, and endomycorrhizal fungi on root morphology in *Pinus*.

By Hugh E. Wilcox, State University College of Environmental Science & Forestry, Syracuse, New York.

It is commonly believed that *bona fide* mycorrhizae in pines are formed principally by invasion of the short-roots by various basidiomycetous fungi. The resultant fungus/root associations develop as ectomycorrhizae which branch dichotomously to produce a wide range of recognizable morphological types. Short-roots not converted to ectomycorrhizae remain uninfected or are invaded by weakly-parasitic fungi which produce anomalous structures subsumed under the cognomen "pseudomycorrhizae". Recently, the universality of this traditional view has been challenged by the widespread prevalence in pines of non-basidiomycetous and non-ectomycorrhizal symbiotic associations. Ectendomycorrhizal infections with large persistent intracellular hyphae, a coarse Hartig net, and little or no mantle are often encountered in coniferous nurseries. In 1965 Mikola isolated from ectendomycorrhizae in Finnish nurseries a non-basidiomycetous fungus designated as the E-strain. Fungi with the characteristics of E-strain are prevalent in nurseries throughout the northern hemisphere, but are by no means the only non-basidiomycetous fungi responsible for ectendomycorrhizae. Ectendomycorrhizae constitute a valid category of root/fungus association and are found not only on seedlings in nurseries but also on wildlings on certain adverse sites. Mycorrhizae syntheses with these fungi show that the root/fungus association is beneficial to the seedling and does not represent an anomalous ectomycorrhizae altered in the direction of parasitism. In fact more tissue damage to roots has been detected in extreme ectotrophs and in those ectotrophs which promote decortication. Ectendomycorrhizal fungi also have less effect than ectomycorrhizae in inhibiting root elongation. The identities of ectendomycorrhizal fungi are largely undetermined, but the large chlamydospores of the E-strain resemble those of the endomycorrhizal fungus *Endogone*. The fact that *Endogone mosseae* produces abundant vescicles in *Pinus resinosa* in pot cultures, indicates that it is not unreasonable to search for the identity of the E-strain amongst the categories of VA mycorrhizae. Thus ectendomycorrhizae are possibly more closely akin to endomycorrhizae in their symbiotic effects than they are to the true ectomycorrhizae. Endophytic invasions of pines have generally been considered parasitic, but there is no reason why this should necessarily be so. An endophytic oomycetous fungus is widespread in thrifty 2-year transplants of *Pinus sylvestris* in the large nursery at Suonenjoki, Finland. Although this fungus develops most vigorously in the various orders of long-roots, it appears to occasionally infect short-roots and produces a recognizable ectendomycorrhiza.

PROGRAM

THIRD NORTH AMERICAN CONFERENCE ON MYCORRHIZAE
HOLIDAY INN, ATHENS, GEORGIA

August 22 - 25, 1977

Monday, August 22

4:00 to 8:00 p.m. - Registration and fee collection

Tuesday, August 23

8:00 to 9:00 a.m. - Registration and fee collection

9:00 to 9:30 a.m. - *WELCOME*, Lauren Coile, President, Athens Chamber of Commerce and Dr. Don Marx, Chairman, 3rd NACOM

9:30 to 10:30 a.m. - *MYCORRHIZAE AND PLANT DISEASES*

Moderator: Dr. Ronald W. Roncadori, Department of Plant Pathology, University of Georgia, Athens 30602

10:30 to 11:00 a.m. - Break

11:00 to 12:00 noon - *MYCORRHIZAE AND PESTICIDES*

Moderator: Dr. Gene R. Safir, Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan 48824

12:00 to 1:30 p.m. - LUNCH ON YOUR OWN

1:30 to 3:00 p.m. - *PRACTICAL APPLICATION OF MYCORRHIZAL TECHNOLOGY*

Session I - Moderator: Dr. A. B. Hatch, P. O. Box 6, Peterboro, New York 13134

3:00 to 3:30 p.m. - Break

3:30 to 5:00 p.m. - *PRACTICAL APPLICATION OF MYCORRHIZAL TECHNOLOGY*

Session II - Moderator: Dr. Charles B. Davey, School of Forestry, North Carolina State University, Raleigh, North Carolina 27607

Wednesday, August 24

8:00 to 11:30 A.M. - *TOUR - Institutes' Mycorrhizal Research Nursery* near *Athens, Georgia*, to view on-going mycorrhizal research on ecto-and endomycorrhizae of forest trees.

11:00 to 1:00 P.M. - LUNCH ON YOUR OWN

1:00 to 2:00 P.M. - *MYCORRHIZAE AND POLLUTANTS*

Moderator: Dr. John A. Menge, Department of Plant Pathology, University of California, Riverside 92502

2:00 to 3:00 P.M. - *FIELD OBSERVATIONS ON MYCORRHIZAL ASSOCIATIONS AND THEIR FUNGI*

Moderator: Dr. Orson K. Miller, Department of Botany, Virginia Polytechnic Institute, Blacksburg, Virginia 24061

3:00 to 3:30 P.M. - Break

3:30 to 5:00 P.M. - *MYCORRHIZAE AND PLANT ECOLOGY*

Moderator: Dr. James Trappe, USDA, Forest Service, Forestry Sciences Laboratory, 3200 Jefferson Way, Corvallis, Oregon 97331

Thursday, August 25

8:30 to 10:00 A.M. - *PHYSIOLOGY OF ENDOMYCORRHIZAL ASSOCIATIONS AND THEIR FUNGI*

Moderator: Dr. James W. Gerdemann, Department of Plant Pathology, University of Illinois, Urbana 61801

10:00 to 10:30 A.M. - Break

10:30 to 12:00 NOON - *PHYSIOLOGY OF ECTOMYCORRHIZAL ASSOCIATIONS AND THEIR FUNGI*

Moderator: Dr. Robert G. Linderman, USDA, ARS, Ornamental Plant Research Lab, 3420 S W Orchard Street, Corvallis, Oregon 97330

12:00 to 1:30 P.M. - LUNCH ON YOUR OWN

1:30 TO 3:00 P.M. - *MYCORRHIZAE AND OTHER SUBJECTS*

Moderator: Dr. Hugh Wilcox, State University of New York, College of Environmental Science and Forestry, Syracuse 13210

3:00 TO 3:30 P.M. - BREAK

3:30 TO 4:30 P.M. - *IMPROMPTU MYCORRHIZAL SUBJECT AREAS*

OPEN SESSION - Moderator: Dr. Robert Todd, Institute of Ecology, University of Georgia, Athens 30602

4:30 TO 5:00 P.M. - *PLANNING FOR FOURTH NORTH AMERICAN CONFERENCE ON MYCORRHIZAE* - WHEN; WHERE; WHO?

Dr. Don Marx, Chairman, 3rd NACOM

6:00 TO 7:00 P.M. - SOCIAL ADJUSTMENT HOUR (CASH BAR) AT HOLIDAY INN. *(LAW PROHIBITS BYOB)*

7:00 TO 8:00 P.M. - BANQUET AT HOLIDAY INN

8:00 TO 8:45 P.M. - *ADDRESS*

Professor J. L. (Jack) Harley, Department of Forestry, Commonwealth Forestry Institute, South Parks Road, Oxford, England OX1 3RB U.K.